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Title: AROMA COMPARISON OF 'MARION' (*Rubus sp.* L.) AND 'THORNLESS EVERGREEN' (*R. laciniatus* L.) BLACKBERRIES

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Michael Qian

'Marion' and 'Thornless Evergreen' blackberry volatiles were analyzed by capillary gas chromatography-flame ionization detection (GC-FID) and GC-mass spectrometry (GC-MS). Based on total percentage of FID area 'Thornless Evergreen' contains significantly more alcohols, hydrocarbons, and phenols than the 'Marion'; 'Marion' contains more acids and esters. Both cultivars contained comparable amounts of aldehydes and ketones; alcohols were most abundant. The six most abundant volatiles in 'Marion' were ethanol, acetic acid, hexanoic acid, ethyl acetate, linalool, and 2-heptanol; they totaled 52% of total peak area. In 'Thornless Evergreen' the six most abundant volatiles were 2-heptanol, ethanol, 2,3-butanediol, hexanol, α-pinene, and ethyl acetate; they totaled 43% of total peak area.

'Marion' and 'Thornless Evergreen' blackberry aromas were compared using a pair of extraction and gas chromatography-olfactometry-mass spectrometry (GC-O-MS) methods. One method is based on purge-and-trap (P&T, dynamic headspace) extraction and aroma intensity rating by detection frequency (DetF) and a numeric scale, and the other based on solvent assisted flavor extraction (SAFE) and aroma threshold dilution analysis (AEDA). The parallel use of P&T-DetF GC-O and SAFE-AEDA provided more representative blackberry volatile compositional data than either alone. Eighty-four compounds were identified; seventy-seven were in 'Marion', and sixty-eight in 'Thornless Evergreen'. Thirty-seven have not been previously

reported in blackberry. Fourteen volatiles out of eighty-four were described with aroma descriptors specific to bramble fruit (berry, blackberry, bramble, raspberry); no single compound was unanimously described as "characteristically blackberry".

Fresh 'Marion' blackberry aroma has been described as floral, fruity, sweet, caramel-fruity, and woody, while fresh 'Thornless Evergreen' aroma is spicy, green, herbaceous, fruity, and sweet. Except for esters, the cultivars contain comparable numbers of acids, alcohols, aldehydes, furanones, hydrocarbons, ketones, phenolics, sulfur, and Theaspirane compounds. Research data implies some portion of the more floral, fruity, and sweet aroma of the 'Marion' blackberry may be the result of additional esters not shared with the 'Thornless Evergreen' blackberry, yet both cultivars apparently contain five furanones, which are powerful sources of sweet, fruity, and spicy aromas. Aroma reconstitution studies will be the key to resolving the significant aroma profile differences between 'Marion' and 'Thornless Evergreen' blackberries, as characteristic blackberry aroma is apparently a complex formulation of volatiles.

AROMA COMPARISON OF 'MARION' (*Rubus sp.* L.) AND 'THORNLESS EVERGREEN' (*R. laciniatus* L.) BLACKBERRIES

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APPROVED:

Major Professor, representing Food Science and Technology

Head of Food Science and Technology

Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Keith Klesk, Author

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CHAPTER 1. INTRODUCTION

BACKGROUND

Food Flavor

The flavor of a food is described by its volatile and non-volatile components, that is, by smell and taste (Lawless and Lee, 1993). The smell of food is composed of "aroma", olfactory sensations from sniffed volatiles, and "odor" (colloquially, "flavor"), olfactory sensations retronasally obtained from volatiles released in the mouth (Acree, 1993; Lawless and Lee, 1993). Generally, much of what is colloquially described as a food's "taste" is actually the flavor derived from the food's volatiles. A food may contain numerous volatiles, but the odor impact of only a small subset of them constitutes the food's characteristic smell (Mistry et al., 1997). The smell of a food may be the result of a complex combination of volatiles, or it may be the result of relatively few compounds, ones described as "character impact compounds" (Mistry et al., 1997). In general, there is no direct relationship between compound concentration or volatility and odor activity (Acree and McLellan, 1993; Teranishi and Kint, 1993). Additionally, there is no obvious relationship between chemical molecular structure, shape, size, and odor activity (Von Ranson et al., 1992; Takeoka et al., 1991, 1995, 1996, 1998). The odor activity of volatile flavor compounds, as measured by their detection thresholds, varies greatly, from parts-per-million (ppm) to parts-per-billion (ppb) levels; for example, in water, 180 ppm for pyrazine, and 0.0001 ppb for 1-*p*-menthene-8-thiol (Van Gemert, 1999; Buettner and Schieberle, 2001a).

Human Olfactory System

While currently proposed mechanisms for human taste are relatively few (Lawless and Lee, 1993; Van der Heijden, 1993), the development of a plausible, consistent mechanism to explain the sensitivity and range of the human olfactory system is hindered by the need to continually integrate extensive, detailed olfactory information from molecular, physiological, imaging, and genetic studies (Firestein, 2001). The current (2001) consensus model of the human olfactory system proposes that an odorant molecule may possess multiple combinations of "numerous" molecular features called "epitopes", or "determinants" (Firestein, 2001). Humans possess approximately 1000 proteinaceous olfactory receptors, and these receptors recognize different "epitopes" (Pickenhagen, 1989; Firestein, 2001). Most odor molecules are recognized by more than one receptor, and most receptors recognize several odors, "probably related by chemical property" (Firestein, 2001). Current experimental evidence suggests that the olfactory receptors have varied sensitivities to odorant molecular features such as chemical functional group and molecular length, among others. Odor recognition (i.e., strength and quality) is then a function of which receptors are activated, and to what extent. This combinatorial strategy allows detection of the enormous collection of odors present (Pickenhagen, 1989; Firestein, 2001). However, this olfactory system shows large variation in acuity among the human population. Normal olfactory sensitivity can range up to 1000-fold between the least and most sensitive observers. Further, approximately 95% of the population has detection thresholds between one-tenth and ten times the mean threshold concentration for a given odorant (Amoore, 1971). These facts complicate the analysis of food flavor compounds.

Volatile Analysis

Gas-chromatography (GC) is the preferred method for volatile compound analysis; in concert with chemical identification and aroma characterization equipment and methods, GC can identify volatile compounds and their odor activity (strength and quality of odor). Mass spectrometry (MS) and chemical standards identify the volatiles, while GC-olfactometry (GC-O) techniques determine which of the volatiles possess odor activity. GC-O methods use human subjects to assess the odor quality of volatiles as they elute from GC columns; the differences in the methods concern the effects of sample preparation, sample replications, and how the data are analyzed (Van Ruth, 2001). Once the significant odor active volatiles are identified, some quantitative measure of them in the original food is required to determine which of them do contribute to the smell of the food. This is a difficult task, as food smell is a complex perception, and compound concentration and odor intensity are not necessarily positively correlated for all human subjects. Further, the sensory impact of odor active compounds in a food matrix may be quite different from their impact when they elute from a GC column (Van Ruth, 2001). The quantification method in use applies measures known as "odor units" (Buttery, 1993), or "odor activity values" (OAV; Buttery, 1993; Grosch, 1994). An OAV is the ratio of compound concentration to compound odor threshold (Grosch, 1994). A compound with an OAV greater than one may contribute to the smell of a food, while an OAV less than one implies the compound does not contribute significantly to the smell of a food (Buttery, 1993). These GC-O and quantitative efforts to assess odor active compounds can be no more than screening methods, as they do not provide an accurate, integrated assessment of the complex chemical and psychophysical interactions that make up the sense of smell.

Blackberries

Wild and cultivated blackberries have been used as food and medicine for hundreds of years (Mazza and Miniati, 1993). Numerous blackberry cultivars have been developed, and currently the predominant cultivar planted is the 'Marion' blackberry (*Rubus sp.* L.) (Strik, 1992; Finn et al., 1997). The flavor of the 'Marion' blackberry is greatly preferred over that of the formerly dominant 'Thornless Evergreen' (*R. laciniatus* L.) (Strik, 1992; Finn et al., 1997). This preference has stimulated blackberry research to correlate quantifiable flavor characteristics to berry genetic makeup, as part of breeding efforts to develop new thornless, winter-hardy blackberry cultivars with 'Marion' flavor. Little aroma research has been done on blackberries, and most blackberry research examined volatile compounds in fresh or processed blackberries (Scanlan et al., 1970; Houchen et al., 1972; Gulan et al., 1973; Georgilopoulos and Gallois, 1987a, 1987b, 1988; Humpf and Schreier, 1991; Herrmann, 1992; Li et al., 1998). Further, although aroma differences between 'Marion' and 'Thornless Evergreen' blackberries have been subjectively described (Finn et al., 1997), no rigorous comparisons have been made.

Research Objectives

The main objective of this research was to identify and compare those volatile compounds that contribute to the aromas of 'Marion' and 'Thornless Evergreen' blackberries. Specific objectives were to identify and compare aroma compounds of the cultivars using two complementary GC-O methods, and to use this data to duplicate each cultivar's aroma profile using OAVs and aroma reconstitution. Additional objectives were to identify and compare cultivar volatile compositions using GC, to gain insights on the phytochemical origins of their flavor compounds and precursors.

LITERATURE REVIEW

Blackberry Use and Origin

A bramble, or caneberry, is defined as any member of the large plant family *Rosaceae*, genus *Rubus* (Bowling, 2000). Extensively cultivated, blackberries (subgenus *Eubatus*) and raspberries (subgenus *Ideobatus*) are the best known brambles (Moore, 1984; Moore and Skirvin, 1990; Bowling, 2000). A versatile fruit, blackberries are consumed fresh, but commercially most are processed into a variety of foods (Moore and Skirvin, 1990; Finn et al., 1997). They are an excellent, low-fat, sodium free, cholesterol-free nutritional source; one serving (144 g) provides 50% of the recommended daily value of vitamin C, 10% of folate, and 22% of fiber. They are also good sources of potassium, calcium, and iron (USDA-ARS, 2002).

Blackberries are a highly heterogenous, heteroploid, interfertile species. Except for desert regions, blackberries are found world-wide; most domestication and commercial use of them has been made in North America (United States) and Europe (Moore and Skirvin, 1990). Many cultivars have been developed, and are grouped based on the presence or absence of thorns, and their manner of growth (Strik, 1992; Bowling, 2000). Thorny erect types, which dominate wild blackberry populations, are cultivated in all six United States growing regions: Mid-Atlantic, Midwest, New England, South Central, South East, and the Pacific Northwest (Bowling, 2000). Thornless semi-erect blackberries are cultivated in four of the six regions (not in New England or the South East), while the (western) trailing blackberries (thorny and thornless) are essentially grown only in the Pacific Northwest (Moore and Skirvin, 1990; Strik, 1992; Bowling, 2000).

The two most economically important trailing blackberry cultivars planted in the Pacific Northwest are 'Marion' and 'Thornless Evergreen'. 'Thornless Evergreen' (*R. laciniatus* Willd., a thornless sport of 'Evergreen'),

was formerly the predominant cultivar planted, but in the early 1980's it was replaced by the 'Marion' (*R. sp.* L.), which is considered to have superior flavor (Strik, 1992; Finn et al., 1997). The 'Thornless' 'Evergreen' blackberry is a periclinal chimera of a selection of *R. laciniatus* ('Evergreen') initially established in North America from Europe. Introduced in 1926, the plants are vigorous and produce medium-sized firm fruit (Moore and Skirvin, 1990; Crandall, 1995; Bowling, 2000) whose aroma has been described as spicy, green, herbaceous, fruity, and sweet (Klesk and Qian, 2003b). The 'Marion' blackberry is a hybrid introduced in 1956; it was selected from a cross between 'Chehalem' and 'Olallie' blackberries. The pedigree of 'Marion' aroma includes at least 5 *Rubus* species: *R. ursinus*, *R. armeniacus* Focke, *R. flagellaris* Willd., *R. aboriginum*, and *R. idaeus* L. (red raspberry) (Finn et al., 1997). 'Marion' plants are very vigorous, and produce medium-large, medium-firm fruit (Moore and Skirvin, 1990) with an aroma described as floral, fruity, sweet, caramel-fruity, and woody (Klesk and Qian, 2003b).

Methodology

Sample Preparation

Analysis of fruit volatiles is difficult, as they typically constitute less than 50 ppm of total fruit mass (Teranishi and Knit, 1993; Takeoka and Full, 1997). Fruit flavors are very complex mixtures, and volatile compounds may be found in all fruit components – water, fats, oils, or carbohydrates. Sample preparation is critical to obtain a representative sample of fruit for analysis. Preparation includes knowledge of how fruit was selected, the duration and type of storage conditions, seasonal and environmental effects, cultivar variations, ripeness, and processing conditions, if any (Teranishi and Knit, 1993). Depending on extraction method goals, the fruit may be left intact, or juiced, puréed, or otherwise processed. It is also prudent to consider enzymatic

and heating effects during sample preparation, as they can alter the actual volatile profile through decomposition or artifact generation (Teranishi and Knit, 1993; Takeoka and Full, 1997). High concentrations of neutral salts (e.g., saturated $CaCl_2$ solutions) have been shown to deactivate fruit enzymes (Buttery, 1987, 1988, 1993). The second phase of sample preparation concerns extraction of flavor volatiles from the fruit matrix. Numerous methods to isolate and concentrate volatiles have been developed but each one alters to some extent the overall volatile composition obtained from the fruit (Zabetakis and Holden, 1997). Generally, fruit volatiles may be solvent extracted, distilled out from the fruit matrix, or collected from the headspace above it.

Solvent Extraction

Solvent extractions utilize organic solvents to extract volatiles from fruit matrices. Typically the fruit is blended, and then either batch or continuously extracted with solvent. Choice of solvent is a function of extraction time, and target volatiles' characteristics (polarity, solubility, etc.). Non-polar solvents such as pentane, hexane, or halogenated hydrocarbons are very effective in rejecting water and low boiling alcohols, while use of diethyl ether will extract more water, methanol, and ethanol (Teranishi and Knit, 1993). Solvent extractions may also pull many non-volatile fruit components into the extract. Fruit lipids and pigment residues can foul GC systems and thermally degrade, producing volatile artifacts (Takeoka and Full, 1997). Solvent extractions are relatively more efficient at extracting low molecular weight acids and very water soluble compounds than distillation methods. Diethyl ether or mixtures of pentane and diethyl ether may be used for extractions; the extracts require drying with suitable drying agents ($MgSO_4$, Na_2SO_4) before concentration (Teranishi and Knit, 1993).

Distillation

Probably the most frequently used distillation method for volatile isolation is simultaneous steam distillation-extraction (SDE) (Teranishi and Knit, 1993; Zabetakis and Holden, 1997; Engel et al., 1999). This method isolates volatiles at 100 °C and 101 kPa (atmospheric pressure) or at 50 °C and 13.3 kPa. The complex glassware has extraction (solvent) and sample sides connected by side arms and a central condenser; a cold finger condenser is used with the central condenser during reduced pressure SDE. The aqueous fruit sample is heated, as is the solvent in the extraction side collection flask. Water vapor and volatiles flow up into the central condenser and meet vaporized solvent. As the solvent and water vapor condense, liquid-liquid extraction of volatiles into the solvent occurs. The liquids collect in their respective side arms, and density differences between solvent and water phases effect the siphoning of solvent plus volatiles and water back into their respective flasks. This distillation-extraction may be run for hours with negligible solvent loss (Teranishi and Knit, 1993). The extract is then dried and concentrated as for solvent extraction. Although this versatile method is one of the oldest used, and extracts are obtained simply and relatively quickly, there is still concern over elevated distillation temperatures that may create volatile artifacts, and alter the true sample volatile composition. Further, SDE discriminates against very water soluble volatiles such as 2,5-dimethyl-4-hydroxy-3-(2H)-furanone (furaneol) (Engel et al., 1999).

To reduce distillation artifact formation, Weurman and others developed a high vacuum transfer (HVT) distillation technique suitable for distillation of a food directly, or a food's solvent extracts (Engel et al., 1999). The method uses an extreme temperature differential between two connected vessels to evacuate the volatiles and leave the non-volatiles behind.

Refinements to the technique led to the development of the solvent assisted flavor extraction (SAFE) distillation unit (Engel et al., 1999). The apparatus consists of a sample dropping funnel, a cooling trap, and a central distillation head bearing two "legs", to which are attached ground glass jointed distillation and recovery flasks. The dropping funnel outlet feeds into the distillation "leg". The vapor inlets to the head and cooling trap are incorporated into the sides of the distillation and recovery "legs", respectively. Sample volatiles are extracted by first thermostatically heating the distillation head and "legs" to the same temperature as that set for the distillation flask water bath. Then high vacuum is applied via the outlet in the cooling trap, and the dropping funnel stopcock is closed. The cooling trap and the Dewars flask surrounding the recovery flask are cooled with liquid nitrogen (Engel et al., 1999). The dropping funnel is filled with sample, and the sample is introduced into the distillation flask at a rate that will not collect liquid sample in the flask. The sample drops vaporize, and the vaporized volatiles and solvent are evacuated into the distillation head, and then into the liquid nitrogen cooled recovery flask, where they condense. Non-volatiles (pigments, fats, carbohydrates) remain in the distillation flask, and the extract can then be dried and concentrated. SAFE produced higher yields of selected model solutions' aromatic compounds from solvent extracts and fatty matrices (50% fat) compared to HVT. The method allows volatiles to be isolated from solvent extracts, aqueous foods such as milk, aqueous food suspensions such as fruit pulps, and even high oil content samples (Engel et al., 1999).

Headspace Analysis

Volatile headspace analysis (HS) is "generally recognized" to produce extracts representative of original food aroma. Further, dynamic HS (DHS), also known as purge-and-trap (P&T), is more efficient than static HS for the

enrichment of trace compounds (Ibáñez et al., 1999). In DHS the fruit sample, which may be intact fruit, pieces, purée, or juice, is placed in a sealed vessel and purged with inert nitrogen gas or purified air. The sample may be stirred while purging, and the purge gas bubbled through the sample or vented just above its surface (Buttery, 1993; Takeoka and Full, 1997). Volatiles are swept up and out of the purge vessel and vented into a trap filled with adsorbent material. The trapped volatiles are then recovered from the trap with a suitable solvent, or by thermal desorption (Buttery, 1993; Takeoka and Full, 1997; Restekcorp, 2003).

There is evidence to support that the choice of purge gas is dependent on the fruit sample species, that is, the sample's metabolism and enzyme suite (Takeoka and Full, 1997). The use of nitrogen (anaerobic conditions) for strawberry foliage DHS analysis yielded greater concentrations of aliphatic alcohols, esters, and aromatics than use of purified air, which yielded higher concentrations of terpene hydrocarbons (Takeoka and Full, 1997). However, studies by Buttery on tomato leaf volatiles showed no significant change in volatile composition with purge gas (Takeoka and Full, 1997). The choice of DHS parameters directly affects which volatiles are isolated, as generally low purge rates of short duration and thermal desorption favor collection of low boiling point volatiles, while high purge flow rates of long duration and solvent elution favor high boiling point volatiles. Purge flow rate and duration must also be weighed against the breakthrough volumes of target volatiles, which may cause volatiles to be swept off the trap before elution or injection. Choice of adsorbents must also be considered, as each varies in adsorption and desorption strength relative to target volatiles and water (Teranishi and Kint,

1993). The adsorbent of choice for fruit volatiles is Tenax®, a porous, hydrophobic polymer (Teranishi and Kint, 1993; Restekcorp, 2003). It is excellent for trapping non-polar compounds, but polar and very volatile compounds are not retained well (Restekcorp, 2003).

Solvent extraction, distillation, and headspace extraction methods share a significant disadvantage common to all extraction methods: they do not completely reproduce the volatile composition of a sample. Each method preferentially discriminates against certain compounds based on solvent or extraction method, so the percentage recovery of volatiles ranges from very low to total recovery (Teranishi and Kint, 1993). Water soluble compounds are poorly recovered with solvent extractions because of poor solubility in organics, but their recoveries by steam distillation are worse because of high water solubility (i.e., low vapor pressures) (Teranishi and Kint, 1993). The choice of DHS parameters can discriminate between low and high boiling point volatiles, polar volatiles, and highly volatile compounds (Teranishi and Kint, 1993; Zabetakis and Holden, 1997; Restekcorp, 2003). Therefore, there is no single ideal volatile sample preparation or extraction method. The complexities of volatile analysis suggest the use of multiple methods to obtain overlapping qualitative and quantitative data that will more accurately represent the true volatile composition in a sample (Takeoka and Full, 1997).

Gas Chromatography

Gas chromatography (GC) is regarded as the forerunner of modern instrumental analysis of volatile organic compounds. The seminal work was published in 1952 (James and Martin, 1952), and the method proved to be simple, fast, and appropriate for the separation of many volatile materials. GC

theories were continually advanced from that point, and led to the mature GC techniques used today (McNair and Miller, 1998). The use of advanced engineering and more robust capillary (open tubular) columns has produced very powerful GC analytical equipment (Braithwaite and Smith, 1996).

Gas-liquid chromatography (GLC, also described as simply GC) is the premier qualitative and quantitative analytical technique for flavor compounds (Teranishi and Kint, 1993; Zabetakis and Holden, 1997; McNair and Miller, 1998). The technique injects a vaporized sample into the end of a heated fused-silica capillary column. A carrier gas (hydrogen, helium, or nitrogen) sweeps the volatiles into the column and onto the stationary liquid phase coating the interior; the liquid phase is cross-linked and covalently bonded to the interior surface of the capillary (Cserháti and Forgács, 1999). The partitioning of volatiles between the carrier gas phase and stationary liquid phase effects volatile separation (Braithwaite and Smith, 1996; McNair and Miller, 1998; Cserháti and Forgács, 1999). Since GC is a separation technique, it does not provide unambiguous identification of unknowns until the column effluent is coupled to an appropriate chemical detector (Mussinan, 1993).

Many different detectors and data analysis equipment have been developed to maximize their sensitivity, selectivity, and ability to quantify volatile sample components (Cserháti and Forgács, 1999). Two types of chemical detectors are extensively used in volatile analysis. The flame ionization detector (FID), considered the universal GC detector, is widely used as it has high sensitivity to virtually all organic compounds, and a good linear detection range over a wide sample quantity range (10^{-3} – 10^{-11} grams injected) (Braithwaite and Smith, 1996; McNair and Miller, 1998). The concurrent use of known and internal standards allows for the identification and quantification

of sample volatiles. While an FID is an excellent general purpose detector, it does not identify volatile peaks per se, so the information it provides is limited compared to that obtained with a mass spectrometer (MS). MS can provide qualitative and quantitative data on a wide range of volatile unknowns (McNair and Miller, 1998; Mussinan, 1993). MS was first coupled to GC in 1959 (Gohlke, 1959), and by the late 1960's dedicated GC-MS systems were being designed to couple the analysis speed and resolution of GC to the qualitative (compound structure, composition, molecular weight) and quantitative capabilities of MS (McNair and Miller, 1998). GC-MS systems are now the preferred choice in volatile instrumental analysis; in selected ion mode (SIM) MS detection limits are 10^{-13} grams, and in full scan mode detection limits are 10^{-9} grams (Mussinan, 1993; Zabetakis and Holden, 1997; McNair and Miller, 1998).

Gas Chromatography-Olfactometry

Once the volatile composition of a fruit has been isolated, separated, and identified via GC analysis, the next step is to determine which of the potentially hundreds of identified volatiles actually contribute to the smell of the fruit. What humans perceive as fruit smell is a complex psychophysical interaction between the human olfactory system and fruit odor active volatiles. Typically the characteristic smell of a fruit is the result of a small subset of the fruit's complex volatile mixture (Grosch, 1994). Individual volatile contributions to that smell are a function of a volatile's potency (odor threshold), concentration, and its perception relative to other odor active volatiles (Takeoka and Full, 1997; Zabetakis and Holden, 1997). Further, the

overall aroma and odor character of a fruit influences the relative importance (i.e., the perception) of a given volatile to that character (Mistry et al., 1997). Finally, human "responses to mixtures of stimuli are characterized by inhibition and suppression, and not by synergy" (Acree, 1993).

It is reported that the human olfactory system has a theoretical odor detection limit of about 10^{-19} moles (Mistry et al., 1997), which is far more sensitive than any physical detector (Pollien et al., 1997). Accordingly, food flavor analysis has generated four olfactometric techniques that use the human nose as an "organic detector". These techniques provide qualitative and arguably quantitative data on odor active volatiles in foods. They are generally described as dilution, time-intensity, detection frequency, and posterior intensity methods (Pollien et al., 1997; Hanaoka et al., 2000; Van Ruth, 2001).

Dilution method

Dilution methods typically use one to three trained assessors to sniff a series of successively more dilute samples until no odors are detected in the GC effluent. Two variants of this method are used: Aroma extract dilution analysis (AEDA) (Ullrich and Grosch, 1987; Grosch, 1994) and "CharmAnalysis" (Acree et al., 1984; Pollien et al., 1997). AEDA was developed in 1987, and measures the maximum relative intensity of odor active volatiles in a sample (Acree, 1993; Hanaoka et al., 2000). A solvent extracted volatile sample is dried and concentrated, and then serial dilutions of 1:2 (or 1:3) are made by addition of solvent, to produce a sequence of 7-10 samples where each member of the series is two (or three) times as concentrated as the next most dilute sample. These samples are then chromatographed and the GC column effluent split between a chemical detector and a sniffing port. As the separated volatiles exit the sniffing port, human subjects sniff the volatiles and record those detected by retention time and odor descriptors (Acree, 1993; Hanaoka et al., 2000). The subjects sniff

the entire dilution series, and the odor activity of each detected volatile is determined from the collected data. For each detected volatile, the greatest dilution (x) at which the odor is still detected is determined. The magnitude of the dilution (2^x, or 3^x) is called a dilution value (DV), or flavor dilution value (FD), and represents the odor intensity of that volatile in the sample (Ullrich and Grosch, 1987; Acree, 1993). The FD value is the ratio of odorant concentration in the initial extract to the odorant concentration in the greatest dilution at which the odorant is still detected; therefore FD values are relative measures (Grosch, 1994). It is assumed that a volatile with a "high" FD value contributes more to the smell of a food (Mistry et al., 1997; Hanaoka et al., 2000). If similar samples (e.g., two blackberry cultivars) are prepared identically, their "comparative AEDA" can be used to identify differences between their odor active volatiles (Mistry et al., 1997; Buettner and Schieberle, 2001b).

Charm analysis was proposed in 1984, and is a "continuous AEDA" dilution method (Acree et al., 1984). Sample preparation is identical to that of AEDA, and the primary difference is that Charm analysis uses computer software to record FD values over the entire time the compounds elute, while AEDA generates only the maximum FD values for compounds (Acree, 1993; Mistry et al., 1997). Where an AEDA output is a single maximum FD value, the corresponding Charm output is peak height (maximum FD value) and area ("Charm value", comparable to a chromatographic peak area) (Guichard et al., 1995; Hanaoka et al., 2000). The measure of odor activity in AEDA is the maximum FD value, in Charm analysis it is the Charm value (Takeoka and Full, 1997; Acree, 1993).

A major criticism of olfactometric dilution methods states that their relative measures of odor activity do not reflect a volatile's true odor contribution to a food (Van Ruth, 2001). Patton and Josephson (1957)

proposed assessment of volatile odor significance in food by relating compound concentration to odor threshold. This concept has several descriptions: "aroma value" (Rothe and Thomas, 1963), "unit flavor base" (Keith and Powers, 1968), "odor unit" (Teranishi et al., 1991), and "odor activity value" (OAV) (Grosch, 1994). OAV is defined as the ratio of compound concentration to its odor threshold (in air, oil, or water) (Grosch, 1994; Mistry et al., 1997). An OAV greater than one means a compound may contribute to the smell of a food (Buttery, 1993). OAVs do not infer anything about stimulus concentration and intensity above threshold but they can indicate the relative importance of odor active compounds. Comparison of OAVs allows food compounds to be ranked according to their probable sensory contributions (Guadagni et al., 1966a, 1966b). OAVs should be considered enhancements to AEDA, and once FD values are determined, odorants with high FD values are quantified with stable isotope dilution assays or chemical standards (Acree, 1993; Grosch, 1994; Mistry et al., 1997), and their OAVs calculated. While there are compilations of odor threshold values for many food aroma compounds, they are not exhaustive. Flavor researchers may be required to experimentally determine odor threshold values for some target aroma compounds.

Although dilution methods have been used since the mid-1980s, additional criticism is leveled at the assumption that odor response is linear to stimulus concentration, and that the linear relationships of all odor active compounds share the same slope (Guichard et al., 1995; Mistry et al., 1997). These assumptions are inconsistent with current psychophysical knowledge. The relationship of odor intensity versus concentration is a power function, a sigmoid curve, with different shapes and slopes for different compounds

(Acree, 1993; Guichard et al., 1995; Hanaoka et al., 2000). This means that compounds with equal thresholds, that is, the same FD values, may display dramatic intensity differences in successive extract dilutions (Guichard et al., 1995; Van Ruth, 2001).

Dilution methods have also been criticized for evaluating odor active compounds "out-of-context". Detractors fault the methods for not fully accounting for odorant losses during isolation, and for basing odor intensity comparisons on only odor thresholds in air, when the actual composition and interfaces of a food matrix may generate complex odor threshold functions (Mistry et al., 1997; Buettner and Schieberle, 1999, 2001a, 2001b). Because of cross-adaptation from a prior eluting compound, a compound may not be detected in a dilution, but is detected in a more dilute sample where cross-adaptation has no effect (Mistry et al., 1997). Further, compound contrast effects alter dilution method results. The elution order, odor character, and perceived intensity of a compound directly influence perceived intensity of a compound eluting after it. Dilution methods do not account for differences between nasal and retronasal odor thresholds and OAVs (Mistry et al., 1997). Dilution methods are time consuming because of the requirement for multiple serial dilutions on multiple GC columns (Guichard et al., 1995; Mistry et al., 1997; Hanaoka et al., 2000). The lengthy time requirements also make it difficult to do sample replicates, and check the reproducibility of assessor results. The length of time used to conduct a single dilution run is also of concern. A dilution run that is "too long" is argued to increase variability in individual GC-O responses due to lethargy, while a run that is "too short" (too rapid) increases variability due to fatigue, adaptation, and sensory saturation of assessors (Mistry et al., 1997).

Time-intensity method

A time-intensity olfactometric method attempts to use the human olfactory system as a "calibrated" organic detector to "quantify" odor active volatiles. Time-intensity methods do not use the multiple extract dilutions of AEDA or CharmAnalysis, but rather use a set of trained assessors to analyze volatiles eluted from a single extract sample (Pollien et al., 1997; Van Ruth, 2001). The single and oldest time-intensity method developed is called *OSME*. *OSME* was developed in 1990 (Miranda-Lopez et al., 1992; Da Silva et al., 1994), and its authors state this time-intensity approach is based on modern psychophysical concepts of odor perception. Stevens' and Fechner's laws are currently accepted as the best representations of sensorial perception properties. Stevens' law has the majority following, and states that odor response to stimulus concentration follows a power function (Stevens, 1961, 1970; Pollien et al., 1997). *OSME* assessors sniff GC effluents, and record detected volatile odor descriptors, duration, and perceived odor intensity based on a 16-point scale, where 0 = not detected, 1 = slight impact (just detected), and 15 = extreme impact (intensity). Assessors are trained and "calibrated" to the 16-point scale with intensity standards, and are trained with potential target compounds to obtain sample familiarity, and a consensus odor descriptor vocabulary (Da Silva et al., 1994).

The seminal paper on *OSME* claimed the method to be "fairly quantitative as compared with traditional olfactometry techniques" (Da Silva et al., 1994), and other literature claimed "OSME stands by itself since the method is based on valid sensory relationships and the intensity data collected are quantitative, permitting statistical comparisons between compounds and samples" (Mistry et al., 1997). *OSME* authors claimed the method was reliable and reproducible, stating that trained human subjects are "reliable instruments" (Da Silva et al., 1994; Pollien, et al., 1997). However, review of the few

published applications of *OSME* indicates the reported assessor reliability occurs only under a specific, restricted set of GC-O conditions (Etiévant et al., 1999). *OSME* authors claimed good, reproducible results for odor active peak intensities, but this claim was based on a model mixture of six pure components well separated by retention indices (Da Silva et al., 1994; Pollien, et al., 1997).

The few published applications of *OSME* show common characteristics of extensive and time consuming training of panelists, but most notably the interpretation of odorant data using a detection frequency method (to be discussed), vice correlation of actual odorant intensities to odorant concentrations, as presented in the *OSME* methodology paper (Da Silva et al., 1994). The 1994 study, using a model mixture of six pure compounds, reported assessors were capable of producing significant ($P \leq 0.05$) relationships between odor intensity (0-15 scale) and odorant concentration, and between aroma peak area and odorant concentration (Da Silva et al., 1994). However, in aroma studies conducted by *OSME* authors on hopped beer and 'Pinot Noir' wines, data analysis was made using a detection frequency method; a similar analysis was presented as *OSME* by Le Guen and others (Miranda-Lopez et al., 1992; Sanchez et al., 1992; Pollien et al., 1997; Le Guen et al., 2000). Consensus odor active peak data was generated by first averaging individual assessor's responses (peak detection at least 50% of the time), then averaging those peak responses over all assessors (detection by at least 75% of assessors) (Miranda-Lopez et al., 1992, Sanchez et al., 1992). This method was modified for analysis done on a corn snack, where consensus data was still obtained by averaging responses, but the criterion for peak detection was modified to those peaks detected at least once over all samples by at least two assessors (Da Silva et al., 1993). Missing detections across the

assessors were rated zero intensity in the averaging process. The authors stated they expected this "*OSME*" data analysis to provide several advantages: to account for assessors' different compound sensitivities, to be less conservative in the inclusion of odorants into an odor profile, to be less liberal in reporting sample odor differences, and to treat "data similarly to other sensory techniques, thus allowing for statistical data analysis" (Da Silva et al., 1993).

The statistical analyses of all these studies show that there is wide variability in sensitivity of the "organic detectors" used, which mutes the contention that they are "reliable instruments". The hopped beer study indicated that assessors needed more training to recognize some odor attributes; assessors also applied the intensity scale differently, in spite of "calibration" with reference standards (Sanchez et al., 1992). The corn snack study reported that differences occurred in snack aroma with time, but the coefficients of variation for the measured attributes varied greatly (15 to 132%), suggesting unacceptable precision from "reliable instruments" (Da Silva et al., 1993).

Odor analysis of 'Pinot Noir' wines was performed in 1990 and 1992 (Miranda-Lopez et al., 1992). The wines analyzed were "distinctly different from the others in odor and taste", but data showed high within and between assessor variation in odor sensitivity, reflecting "day-to-day variations in sensitivity" (Miranda-Lopez et al., 1992; Pollien et al., 1997). The use of detection frequency analysis of this *OSME* data has been questioned, and the use thought to be a result of previously reported large inconsistencies, that is, variability, in the number and quality of odorants detected between assessors. It was also thought that "non-*OSME*" analysis of data in this manner implies *OSME* analysis of wine was too difficult a task for assessors to perform (Etiévant et al., 1999).

Two variants of the time-intensity (*OSME*) method have been examined. One records intensity by movement of a computer mouse along a fixed scale (Guichard et al., 1995; Delahunty et al., 1996), while the other records intensity with a cross-modality method of intensity to finger span (Guichard et al., 1995; Etiévant et al., 1999). Both variant methods used model odorant solutions; the finger span method used a trained panel, while the computer mouse method used untrained assessors. Reported results imply trained panels "guarantee better results" using reference compounds. However, results showed high within and between assessors variation in odor sensitivity similar to that reported in the 'Pinot Noir' studies; investigators therefore recommended the use of large (multiple assessor) panels (Miranda-Lopez et al., 1992; Guichard et al., 1995; Pollien et al., 1997; Etiévant et al., 1999). It was noted that an assessor will find it difficult to simultaneously detect an odor, assign a descriptor, and register intensity from a memorized scale. Such effort is exacerbated if volatile peaks elute or coelute rapidly (Pollien et al., 1997).

A paper describing weighted statistical analysis of hop aroma in beer used what is essentially a "static *OSME*" method. A panel of 10 trained assessors evaluated aroma intensity of three treatments of hopped beer against an unhopped control. Although the samples were evaluated directly, and not extracted and separated with GC, their sensory analysis matched that of the *OSME* method with regards to required training of assessors, consensus descriptors, the 16-point intensity scale, and the use of aroma intensity standards (Yang et al., 1994). The investigators noted that to increase the panel's ability to discriminate between treatments, individual assessor sensory ratings needed to be weighted, as their olfactory sensitivity and consistency of analysis varied widely for a given aroma. Using the three statistical measures of F ratio, the corresponding confidence coefficient S, and the correlation coefficient r, three weighting factors (F, S, S x r) were tested in the analysis of

hopped beer aroma. Analysis of variance showed data weighting reduced the effect of less reliable sensory scores from insensitive assessors, and enhanced discrimination of sample differences (Yang et al., 1994). Although the results demonstrate the effectiveness of weighting sensory data in emphasizing treatment differences, the analysis effort expended for eleven beer attributes clearly expands by orders of magnitude for the analysis of a complex GC-O extract.

The use of time-intensity methods to date suggests the actual reproducibility of the method is unknown, but heavily dependent on the reliability of the human assessors used. Further, it is unknown how time-intensity parameters generally relate to physical concentration (Van Ruth, 2001). Finally, the requirement for statistical weighting of sensory panel data to increase panel olfactory sensitivity and reliability is a theoretical necessity, but is impractical for current GC-O analysis of odor active volatiles.

Detection Frequency method

The detection frequency method was proposed by Linssen and others, and relates the odor intensity of a volatile to the number of experienced assessors detecting it, either simultaneously in the same GC-O run (via multiple sniffing ports), or from identical GC-O runs (Linssen et al., 1993; Pollien et al., 1997; Van Ruth, 2001). Dummy samples can be used to determine panel olfactory noise, the signal-to-noise level of the assessors. The method is robust, as demonstrated by the aroma analysis of rehydrated French beans (Van Ruth et al., 1996a). The use of different sampling times in a model mouth system produced identical sets of odor active volatiles (signals above the noise level of the panel), even though the sampling times produced varied quantities of volatiles and numbers of experienced assessors detecting the volatiles (Van Ruth et al., 1996a). The method has been used to identify odor active volatiles in many foods: dried French beans, dried bell peppers, and

dried leeks (Van Ruth et al., 1995a), vegetable oils and emulsions (Van Ruth et al., 1999), lovage (Bylaitė et al., 2000), and mineral water (Linssen et al., 1993), among others. Using this method "significant correlations" were shown between the number of assessors perceiving odor active volatiles and the intensity scores of these volatiles' attributes in parallel but independent sensory analysis (Van Ruth et al., 1995b; Van Ruth et al., 1996b). Additionally, other studies showed the number of assessors perceiving odor active volatiles correlated "very well" to the volatiles' sensory intensity at elution (van Ruth et al., 1996a, 1996b). A similar method by Pollien and others reported "satisfactory" repeatability of results using untrained assessors (Pollien et al., 1997). Using model and real volatile mixtures, researchers demonstrated a compromise between panel reliability and minimum number of assessors required (ideally eight to ten). Using six assessors, two independent untrained panels generated similar results. Pollien's method appears to be more reliable than other GC-O methods, all which require trained assessors (Pollien et al., 1997). Although the literature demonstrates the applicability of this method as an olfactometric measure of odor active volatiles, some consider it a drawback that it is not based on real odor intensities (Van Ruth, 2001).

Posterior Intensity method

The posterior intensity method is infrequently used and vaguely described. The method involves rating odor intensity of volatile peaks after they elute from the GC column. Assessors may be trained in sensory descriptive analysis, but are instructed to use an ordered scale to rate odor intensity (Cormier et al., 1991; Van Ruth et al., 1996b; Van Ruth, 2001). Using a reference mixture of eight volatiles, data from AEDA, detection frequency, and posterior intensity methods were compared. Posterior intensity data correlated "reasonably" well with that of detection frequency ($R = 0.822$),

but less so with AEDA (R = 0.667) (Van Ruth, 2001; Van Ruth and O'Connor, 2001a). However, large variability was noted between assessors (van Ruth, 2001, Drawert and Christoph, 1984). The few applications of the method included evaluation of cheddar cheese aroma (Arora et al., 1995), light-activated milk (Cadwallader and Howard, 1998), and dried French beans (Van Ruth et al., 1996b). In theory, because assessors differ in their use of the ordinal scale, their variation could be reduced by anchoring scale ends with reference odors, to produce a "calibrated scale" similar to that used in *OSME* (Van Ruth, 2001). In fact, a "calibrated posterior intensity" method may be considered another form of "static *OSME* ".

The development of GC-O for the analysis of food odor active volatiles is a logical extension of earlier GC separation and analysis of volatiles. It is well documented that the human olfactory system can be a much more sensitive detector than currently available electronic detectors (Mistry et al., 1997; Pollien et al., 1997). However, the fundamental weakness of all GC-O methods is that "they do not account for interactions arising in the olfactory system or between taste and smell" (Blank, 1997). The human sense of smell is a complex psycho-physiological function that couples highly variable olfactory acuity to stimulus functions of odor active volatiles. These functions are similar, as per Steven's Law, but vary widely with respect to their rates of change of perceived intensity with concentration. These considerable sources of variability are major causes for the inherent lack of repeatability and reproducibility in sensorial techniques (Pollien et al., 1997; Dattatreya et al., 2002).

Besides the previously discussed analytical issues unique to each GC-O method, the methods share some analytical concerns. Generally, analytical conditions and assessors' qualities should be optimized for GC-O analysis. The effective odorant concentration delivered to the sniffing port is a function

of the sample itself, supplemental air flow rate, and GC operating conditions, all of which affect sniffing port chromatographic peak shapes and heights (Van Ruth and O'Connor, 2001b). Peak shape and height in turn affects odor perception, as human smell responds more to a stimulus change than stimulus magnitude (Acree, 1993). GC separation conditions (column type, oven temperature program) affect co-elution, retention time, and resolution of volatiles. These separation characteristics directly affect an assessor's olfactory analysis by either overwhelming the ability to resolve and identify co-eluting or rapidly eluting volatiles, or by altering peak shape and height, as previously discussed (Van Ruth, 2001).

Although studies have recommended short (25 minutes or less) GC-O runs to avoid assessor fatigue (Acree, 1993; Pollien et al., 1997), others found no significant fatigue effects during 45 minute GC-O runs (Van Ruth and O'Connor, 2001b). The use of humidified supplemental air at the sniffing port is generally applied in GC-O, but flow rates vary, and the use of humidification was not firmly justified (Hanaoka et al., 2000). Studies with test solutions showed optimizing air flow increased odor detection frequency and intensity rating, while humidification was unnecessary, as the nose is an efficient natural humidifier of inspired air (Hanaoka et al., 2000). The use of trained panels is supported in the literature as a means to offset wide variation in assessors' olfactory acuity, and to standardize odor descriptors (Mistry et al., 1997; Etiévant et al., 1999; Bylaitė et al., 2000; Serot et al., 2001). However, all panels trained or not, display high variability of intensity measures within and between assessors (Pollien et al., 1997; Etiévant et al., 1999). Effectively no testing has been done to examine effects of training on the quality of assessors' intensity estimates (Etiévant et al., 1999). However, a single reference

examined GC-O training effects on assessor performance. Using a test mixture, researchers reported that assessor training did not affect the detection of odor active volatiles, but did reduce noise levels (Van Ruth and O'Connor, 2001b).

Sensory (psychophysical) measurements are replete with individual human differences. As previously discussed, and regardless of olfactometric method used, cross-adaptation and compound contrast effects may alter the perceived intensity and odor character of volatiles (Mistry et al., 1997). Varying human sensitivity to odorants is a function of age, gender, genetic endowment, menstrual status, and life experiences (Doty et al., 1984; Stevens, 1991). These olfactory sensitivity variations manifest themselves as consistently reported assessor differences in detection thresholds, and the generated exponents of odor intensity power functions of odor active compounds (Berglund et al., 1971; Piggot and Harper, 1975; Tuorila, 1981; Mistry et al., 1997). Such differences directly affect the variability and reliability of intensity measurements made with GC-O (Mistry et al., 1997). It is for this reason that in GC-O analysis a panel of assessors (ideally eight to ten) is a prerequisite, regardless of method used (Pollien et al., 1997; Van Ruth and O'Connor, 2001a).

Detractors of the four GC-O methods discussed make legitimate claims concerning the effects of sample preparation and replications, number of assessors, assessor fatigue, and evaluation of odor active volatiles "out of context" on the volatile analysis of a food (Mistry et al., 1997). However, the dominant contention between methods concerns the appropriateness of the basis used to measure odor intensity. In spite of infrequent use of *OSME* as designed, the literature implies this method is theoretically more acceptable because it is based on current concepts of psychophysics, and measures "real" intensities (Da Silva et al., 1994). Dilution methods are described as

"screening methods" because their assumptions are not psychophysical, but based on odor detection thresholds (Grosch, 1994; Guichard et al., 1995; Buettner and Schieberle, 2001a). The detection frequency method is not psychophysically based, but overcomes limitations from the number of assessors used and the use of detection thresholds by correlating the number of assessors detecting an odor to the odor's intensity. The infrequently used posterior intensity method has not been validated with respect to its relationship with volatile compound concentration, but has been used in various aroma evaluations (Van Ruth, 2001).

Since all the GC-O methods discussed are significantly affected by the wide variability in sensitivity and reliability of the assessors used, and by the samples they analyze, samples which vary in the accuracy with which they represent the true volatile composition being examined, it can be argued that all these methods are no more than screening methods for the detection of potent odorants in food. All intensity measures obtained in GC-O are generally useful, and are approximations of the sensory relevance of odorants (Guichard et al., 1995; Blank, 1997). Regardless of method, GC-O results give indications of the potency of odor active volatiles, but not final conclusions of their sensory relevance (Blank, 1997). GC-O methods do evaluate odorants out of context, as the actual smell of a food is a complex function of the nasal and retronasal stimuli generated from the food matrix. Accordingly, the analysis of food smell must include the analytical quantification of all odor active volatiles suspected to be significant to that smell (Grosch, 1994; Buettner and Schieberle, 2001a, 2001b). The quantifications of these suspect volatiles are done with the same samples used for GC-O, and the data used to perform aroma reconstitution studies (Buettner and Schieberle, 2001a, 2001b; Ferreira et al., 2002), which are the best, most efficient use of trained sensory panels.

Aroma reconstitution produces formulations of suspect odor active volatiles that are compared to the original food sample. By repeated testing and alteration of formulations, panels of trained assessors can develop and identify an aroma formulation that matches original food aroma sensory attributes. Aroma reconstitution studies verify collected analytical data, and corrects for the limitations of GC-O intensity and threshold determinations (Mistry et al., 1997). In view of the costly and time-consuming training and statistical analysis required to establish a suitably sized panel of trained assessors, a panel capable of producing reliable and reproducible results, it seems prudent, cost-effective, and conceptually acceptable to reserve such cost and effort for the final aroma analysis step of reconstitution. Production of the initial list of suspected odor active volatiles should use a more rapid, less costly GC-O screening method. Therefore, in the analysis of odor active volatiles the detection frequency method is best for the initial screenings, and the use of *OSME* as *designed* (i.e., with required statistical weighing of real intensity data) best for the reconstitution.

The Smell of Blackberries

Wild and cultivated blackberries have been used as food and medicine for hundreds of years, and consumer demand for blackberries as juice, concentrate, jellies, jams, and ingredients in a wide variety of foods has generated large scale cultivation of them (Latrasse, 1991; Mazza and Miniati, 1993). Blackberries are prized for their color, flavor, nutritional content, and aroma, but the analysis of blackberry flavors has been limited compared to other small fruits such as raspberry and strawberry (Honkanen and Hirvi, 1990; Shamaila et al., 1993; Zabetakis and Holden, 1997; De Ancos et al., 2000; Hakala et al., 2002). Independent analyses were made on anthocyanins (Mazza and Miniati, 1993), sugars and acids (Wrolstad et al., 1980; Plowman,

1991), and volatile and bound volatile compounds in fresh or processed blackberries (Scanlan et al., 1970; Houchen et al., 1972; Gulan et al., 1973; Georgilopoulos and Gallois, 1987a, 1987b, 1988; Humpf and Schreier, 1991; Herrmann, 1992; Li et al., 1998). Six of the volatile studies (1970, 1972, 1973, 1987a, 1987b, 1988) analyzed 'Evergreen' or 'Thornless Evergreen', but little aroma research had been done. However, no volatile or aroma research has been done on 'Marion', the premier blackberry cultivar planted today. A hybrid blackberry, its pedigree contains at least 5 *Rubus* species, including raspberry (Finn et al., 1997).

The earliest studies of blackberry aroma (1970, 1972, and 1973) examined commercial essences of 'Evergreen'. Scanlan, Houchen, and others (1970, 1972) identified sixteen compounds, in particular 3,5-dimethoxyallyl-benzene (musty odor). Gulan and others (1973) identified six compounds, plus an unknown with an apparent molecular weight of 168 that displayed a strong "blackberry-like odor". The seminal research on blackberry aroma was done on fresh and heated blackberry juices (Georgilopoulos and Gallois, 1987a, 1987b), as well as on a commercial concentrated blackberry juice (Georgilopoulos and Gallois, 1988). Fresh juice from 'Thornless Evergreen' blackberries was solvent extracted with trichlorofluoromethane and dichloromethane. The extracts were analyzed using GC-MS, infrared analysis, and odor measurement. One hundred thirty-five aroma volatiles were identified, and one hundred and ten tentatively so. Thirteen volatiles represented 67% of the total odorous profile, and are thought to contribute to the aroma of fresh blackberries. In order, the major volatiles were 2-heptanol (herbaceous, earthy, 43.06%), *p*-cymen-8-ol (musty, celery-like, 3.72%), 2-heptanone (spicy, fruity, 3.32%), 1-hexanol (herbaceous, sweet, 3.05%), α-terpineol (pine oil, citrus, 2.38%), pulegone (mint, camphor, 2.05%), 1-octanol (fatty, citrus, 1.83%), isoborneol (piney, camphoraceous, 1.76%), myrtenol

(minty, woody, 1.28%), 4-terpineol (musty, dusty, 1.21%), carvone (herbaceous, peppermint, 1.14%), elemicine (woody, floral, 1.12%), and 1-nonanal (floral, citrus, 1.01%) (Georgilopoulos and Gallois, 1987a; Honkanen and Hirvi, 1990).

Georgilopoulos and Gallois also compared volatiles in the heated juices of 'Himalaya' from France and Spain to those of 'Thornless Evergreen' (Georgilopoulos and Gallois, 1987b). The samples were brought to a boil, filtered, and the resultant juices extracted serially by SDE and solvent extraction with dichloromethane. The extracts were analyzed as for the fresh blackberry juice using GC-MS, infrared analysis, and odor assessment. One hundred and ninety-one volatiles were reported. Further, using similar extraction and analysis methods, these researchers analyzed the volatiles in a commercial concentrated 'Thornless Evergreen' juice. Seventy volatiles were reported (Georgilopoulos and Gallois, 1988).

Table 1.1 summarizes the fresh and heated blackberry juice volatile studies of Georgilopoulos and Gallois (1987, 1988); based on the thirteen most abundant volatiles they reported in fresh juice. In general, the heated 'Himalaya' has smaller amounts of the thirteen volatiles than the heated 'Thornless Evergreen', and heating effects were most extreme in the commercial concentrated juice.

Table 1.1: Relative peak area[a] percentage of blackberry volatiles

	'Thornless Evergreen'			Heated 'Himalaya'	
Volatile	Fresh	Heated	Commercial	France	Spain
2-heptanol	43.06	23.07	0.19	0.63	1.70
p-cymen-8-ol	3.72	4.32	0.02	0.25	1.53
2-heptanone	3.32	1.12	0.03	0.08	2.30
1-hexanol	3.05	2.88	-	0.81	5.03
α-terpineol	2.38	3.22	0.19	0.57	0.55
pulegone	2.05	3.07	-	1.23	1.10
1-octanol	1.83	0.31	-	-	1.73
isoborneol	1.76	1.69	-	-	0.07
myrtenol	1.28	1.04	-	0.20	0.09
4-terpineol	1.21	2.70	-	0.12	0.29
carvone	1.14	0.47	-	-	-
elemicine	1.12	0.09	-	trace	-
1-nonanal	1.01	4.94	-	1.97	1.36

[a] relative to total peak area

It is interesting to note that the commercial concentrated juice was described as having a "rather pleasant and characteristic blackberry aroma" (Georgilopoulos and Gallois, 1988), while multiple attempts to reproduce fresh blackberry aroma with mixtures of pure samples of the most abundant volatiles (Table 1.1) resulted in an odor profile "somewhat reminiscent of blackberries, but lacking the delicate aroma of the natural extract" (Georgilopoulos and Gallois, 1987a).

Table 1.2 summarizes identified volatiles by compound class in fresh and heated 'Evergreen' cultivars (Nijssen et al., 1996). Although fewer volatiles (118) were reported in the heated juice than in the fresh (147), the total number of volatiles in seven out of twelve compound classes is essentially

unchanged between the fresh and heated fruit. However, the heated juice did show thermal effects by having significantly fewer esters, ketones, hydrocarbons, and lactones, but a higher number of furans, than the fresh juice.

Table 1.2: Blackberry volatile percentage

Volatile class	'Evergreen'[a] Fresh	Heated
Alcohols	22.45 (33)	24.58 (29)
Esters	22.45 (33)	14.41 (17)
Aldehydes	18.37 (27)	22.88 (27)
Ketones	14.28 (21)	11.02 (13)
Hydrocarbons	7.48 (11)	4.24 (5)
Lactones	7.48 (11)	5.93 (7)
Phenols	3.40 (5)	3.39 (4)
Acetals	1.36 (2)	3.39 (4)
Acids	1.36 (2)	0.00 (0)
Furans	1.36 (2)	7.63 (9)
Ethers	0.00 (0)	0.85 (1)
(Ep)oxides, pyrans, coumarins	0.00 (0)	1.70 (2)

[a] %, (number of compounds)

Although blackberry fruit contains a significant number of odor active volatiles, no single one has been conclusively identified as a "character impact compound", with an aroma described as "characteristically blackberry". The data presented implies that blackberry aroma is the result of a complex formulation of volatiles, which includes minor components that provide the subtle background scents that refine and balance overall blackberry aroma (Georgilopoulos and Gallois, 1987a; Latrasse, 1991).

Blackberry Volatile Metabolism

The biogenesis of fruit volatiles is of interest to flavor chemists, plant breeders, and biotechnologists alike, as identifying the phytochemical origins of flavor compounds and their precursors provides insights on the composition and proportions of those fruit volatiles that define fruit smell. This phytochemical knowledge is used in parallel by these disciplines to identify, develop, and enhance consumer-preferred olfactory qualities of fruit. Fruit flavor research has examined free volatiles and bound glycosidic precursors (i.e., bound volatiles) in grapes, fruit juices, and wine (Rouseff and Leahy, 1995). Research studies have examined volatile biogenesis in apples, kiwi, pineapple, strawberry, and tomato, and exotic fruits such as quince, passion fruit, and guava, et al. (Williams, 1993; Rouseff and Leahy, 1995). However, blackberry volatile metabolism has not been specifically addressed. Volatile biogeneration in plants uses three main chemical compound classes: fatty acids, amino acids, and carbohydrates.

Fatty acids are thought to be the primary precursors of most plant volatiles, and in general are broken down by two oxidative pathways: β-oxidation and lipoxygenase (LOX) (Sanz et al., 1997). Beta-oxidation is thought to produce "primary aromas", those generated in intact fruits. Enzymes in the β-oxidation cycle metabolize fatty acid acyl-CoA derivatives to shorter chain acyl-CoAs. The oxidation cycle involves, in order, acyl-CoA dehydrogenase (with FAD), enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase (with NAD), and acetyl-CoA acetyltransferase (thiolase, with free CoA). This enzymatic series generates acetyl-CoA and an acyl-CoA shorter by two carbons. The various resulting acyl-CoAs are converted into esters via alcohol acyltransferase (Paillard, 1979).

Unsaturated fatty acids require auxiliary enzymes to complete oxidation. In the case of a C_n unsaturated fatty acid-CoA, β-oxidation yields a C_{n-x1} (Z)-3-enoyl-CoA ($x1$ = number of carbons removed by oxidation down to the first double bond), which is isomerized by enoyl-CoA isomerase to C_{n-x1} (E)-2-enoyl-CoA . (E)-2-enoyl-CoA is the natural substrate for enoyl-CoA hydratase (Sanz et al., 1997). In the case of polyunsaturated fatty acids, the oxidation is similar to that for an unsaturated fatty acid, but produces a C_{n-x2} (Z)-2-enoyl-CoA ($x2$ = number of carbons removed by oxidation down to the last double bond). This molecule is hydrated by enoyl-CoA hydratase to R-(-)-3-hydroxyacyl-CoA, not the (S)-(+)-enantiomer produced from saturated fatty acids. Conversion to the (S)-(+) isomer, which is the natural substrate of 3-hydroxyacyl-CoA dehydrogenase, is made via 3-hydroxyacyl-CoA epimerase (Sanz et al., 1997). It is thought these two auxiliary enzymes may account for the different enantiomeric ester compositions in tropical fruits (Tressl et al., 1985).

The lipoxygenase (LOX) pathway is thought to produce "secondary aromas" from the disruption of plant tissues, either from crushing, slicing, and the like, or from fruit ripening (Sanz et al., 1997). The LOX pathway generates the "green" aromas in plants, and is preceded by the action of acylhydrolases, which free polyunsaturated fatty acids from glycolipids, phospholipids, or triacylglycerols. LOX degradation of linoleic and linolenic acids generates many fruit acids, alcohols, aldehydes, and esters (Stone, et al., 1975; Olias et al., 1993; Perez et al., 1999). LOX degradation of linoleic and linolenic acids proceeds first via LOX isozymes to produce fatty acid hydroperoxides, preferentially at C_9 or C_{13}, or non-specifically at either carbon. In turn these hydroperoxides are converted to aldehydes and oxoacids via hydroperoxide lyase (HL) (Sanz et al., 1997).

Three classes of HL, C_9, C_{13}, or non-specific, determine aroma composition in many plants, despite the specific action of LOX present. For example, pear LOX forms mainly 13-hydroperoxides, but pear HL is the C_9 class. Olive LOX is the C_9 class, but its HL is the C_{13} class. This specificity of olive HL is evidenced by the almost total absence of C_9 carbonyls, and high content of C_6 alcohols, aldehydes, and esters in virgin olive oil aroma volatiles (Olias et al., 1993). Similarly, cucumber LOX produces C_9: C_{13}: non-specific hydroperoxides in the ratio of 13 : 9/85 : 15, and its HL is non-specific, which accounts for the presence of C_9 carbonyls important to cucumber aroma (Galliard et al., 1976; Wardale and Lambert, 1980).

Hydroperoxide lyase activity on 13-hydroperoxylinoleic acid or 13-hydroperoxylinolenic acid produces 12-oxo-(9Z)-dodecenoic acid and hexanal or (3Z)-hexenal respectively. HL activity on the corresponding 9-hydroperoxides of these fatty acids yields 9-oxononanoic acid and (3Z)-nonenal or (3Z, 6Z)-nonadienal respectively (Sanz et al., 1997). Most plants isomerize compounds with a (3Z)-enal structure to the (2E)-enal form with a (3Z, 2E)-enal isomerase (Sanz et al., 1997). In most plants, the unsaturated aldehydes produced by HL are reduced by alcohol dehydrogenase to their corresponding alcohols, either before or after isomerization. These alcohols are natural substrates for alcohol acyltransferase, to produce esters (Sanz et al., 1997).

In fruits, amino acids are direct precursors of volatile compounds, and when metabolized generate aliphatic, aromatic, or branched acids, alcohols, carbonyls, and esters (Sanz et al., 1997). It has been shown that variations in free amino acid content occur during fruit ripening, when characteristic aroma is produced. This implies that different fruit aroma profiles could be related to a free amino acid pool (Tressl and Drawert, 1973). Amino acids are transformed using three enzymatic classes: aminotransferase, decarboxylase,

and alcohol dehydrogenase. The first step uses an amino acid-specific 2-oxoglutarate aminotransferase to produce a 2-oxoacid from the amino acid. The decarboxylation of the 2-oxoacid is thought to occur via either an enzymatic complex similar to that of pyruvate dehydrogenase (decarboxylating), or 2-oxoglutarate dehydrogenase from the Krebs cycle (Sanz et al., 1997). The various aldehydes produced may then be transformed into alcohols or acids with alcohol dehydrogenase or aldehyde oxidase, respectively. Acyl-CoA products from the action of 2-oxoglutarate dehydrogenase are transformed into esters via alcohol acyltransferase. Evidence exists to indicate decarboxylation final products may depend on the plant species (Sanz et al., 1997).

A different volatile metabolic pathway has been proposed using aromatic amino acid (tyrosine and phenylalanine) precursors, leading to compounds with phenolic and spicy odors. Cinnamic acid, derived from phenylalanine via phenylalanine ammonia lyase (PAL), and *p*-coumaric acid, derived from tyrosine via PAL, or from cinnamic acid via cinnamic acid hydrolase, are suggested as starting intermediates for this pathway (Tressl and Albrecht, 1986; Sanz et al., 1997). Cinnamic acid, through loss of acetate, leads to benzoic acid and its derivatives, while *p*-coumaric acid, converted to caffeic acid by phenolase, leads to phenolic derivatives (Sanz et al., 1997).

While fruit aromas are predominantly based on ester composition, fruits may also use amino acid substrates similarly as vegetables to produce sulfur-containing volatiles with aromas that are vegetal rather than fruity. Free amino acids are indirect precursors of vegetal aromas, as they are metabolized into derivative compounds that in turn are enzymatically converted to aroma compounds with cell disruption (Chin and Lindsay, 1994). Two of the major classes of these compounds are the S-alk(en)yl-cysteine sulfoxides and

glucosinolates. The S-alk(en)yl-cysteine sulfoxides are precursors to the characteristic aroma of *Allium* and *Brassica* species. A proposed pathway suggests cysteine and serine as precursors for the sulfoxides, and the key enzymatic step in aroma generation is accomplished by alliinase (alliin alkyl-sulfenate lyase). Upon cell disruption S-alk(en)yl-cysteine sulfoxides in the cytoplasm are split by alliinases released from vacuoles to produce dialk(en)yl thiosulfinates. These thiosulfinates are unstable and undergo rapid spontaneous non-enzymatic reactions to form numerous volatile sulfurous compounds characteristic of *Allium* and *Brassica* species (Sanz et al., 1997).

Glucosinolates are sulfur compounds whose breakdown products contribute to the flavor of the *Cruciferae* family of plants, but little is known about their biosynthesis. Plants that contain glucosinolates also contain enzymes that degrade them. These enzymes, thioglucoside glucohydrolases, catalyze the hydrolysis of the thioglucosidic linkage in glucosinolates. The released aglucones undergo non-enzymatic reactions to produce volatiles. It is assumed that enzymes and substrates are segregated from one another until cell disruption, as for the S-alk(en)yl-cysteine sulfoxides (Sanz et al., 1997).

Relatively few aroma compounds derive from carbohydrates. Fruit terpenes (mainly monoterpenes) are produced from carbohydrates through the isoprenoid pathway (Sanz et al., 1997). Mevalonic acid is considered the first specific terpene precursor, and is used to produce isopentyl diphosphate (IPP), the hypothetical 'active isoprene' unit from which all isoprenoid compounds derive. IPP is produced by the sequential double phosphorylation of mevalonic acid by mevalonate kinase and 5-phosphomevalonate kinase to produce mevalonic acid diphosphate (MVAPP). IPP is then produced by the decarboxylation and dehydration of MVAPP by MVAPP decarboxylase (Sanz et al., 1997). In order to produce geranyl diphosphate (GPP), the direct

precursor of monoterpenes, one molecule of IPP is isomerized to the dimethylallyldiphosphate form (DMAPP) by isopentenyl diphosphate isomerase. Prenyltransferases then produce GPP by the condensation of DMAPP and IPP. Monoterpenes are then produced from GPP through hydrolysis, cyclations (key step), and oxidoreductions (Sanz et al., 1997).

Furanones are another carbohydrate derived compound class important to fruit aromas. These compounds are the result of the Maillard reaction, the browning reaction of reducing sugars with amine salts (Schwab, 1998; Sanz et al., 1997). Despite the importance of furanones in fruit aroma, their biosynthesis is unclear. Studies were attempted to detail the formation pathway of 2,5-dimethyl-4-hydroxy-3-(2H)-furanone (furaneol), identified as an important aroma in many fruits including pineapple, mango, grapefruit, tomato, strawberry, raspberry, and blackberry (Sanz et al., 1997; Klesk and Qian, 2003b). A study demonstrated furaneol and its derivatives mesifurane (2,5-dimethyl-4-methoxy-3-(2H)-furanone) and furaneol acetate (2,5-dimethyl-4-acetoxy-3-(2H)-furanone) were formed by direct conversion of D-fructose in a biological Maillard reaction (Schwab, 1998). Stable isotope ratio analysis suggests a pathway that converts D-fructose to 1-deoxyfructose or 6-deoxyfructose, which are in turn converted to furaneol, probably through dehydration and reduction reactions. This is contrary to an earlier proposal that furaneol is formed by the coupling of two C_3 units (Schwab, 1998).

Some final comments address ester formation in fruits. Esters constitute the main group of compounds identified in fruit aroma, and are produced by the esterification of alcohols and carboxylic acids. Biogeneration of these precursor alcohols and acids are generally well explained by the enzymatic actions on lipids and amino acids previously discussed. However,

little is known about the actual esterification reaction itself. In micro-organisms two enzymes are implicated in ester formation: alcohol acyltransferase (AAT) and esterase. AAT catalyzes the transfer of an acyl moiety of an acyl-CoA onto the corresponding alcohol, while esterase hydrolyzes esters. These enzymatic activities have been described in fruits (Sanz et al., 1997). Research information indicates that major factors in ester biogeneration include fruit ripening, availability of substrates, and the substrate specificity of alcohol acyltransferases for both the acyl moieties of acyl-CoAs, and the corresponding alcohols (Sanz et al., 1997).

CHAPTER 2.

PRELIMINARY AROMA COMPARISON OF 'MARION' (*Rubus sp.* L.) AND 'THORNLESS EVERGREEN' (*R. laciniatus* L.) BLACKBERRIES BY DYNAMIC HEADSPACE/OSME TECHNIQUE

KEITH KLESK AND MICHAEL QIAN

Journal of Food Science

525 W. Van Buren St., Suite 1000, Chicago, ILL, 60607

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ABSTRACT

'Marion' and 'Thornless Evergreen' blackberry aromas were analyzed with a purge-and-trap gas chromatography-olfactometry/mass-spectrometry (GC-O/MS) technique. Fifty-eight aromas were identified; 30 were common to both cultivars, and 22 have not been previously reported in blackberry fruit. Comparison of cultivars shows the 'Marion' blackberry contains more esters, while the 'Thornless Evergreen' contains more alcohols. The aroma profile of blackberry is complex, as no single volatile was unanimously described as characteristically blackberry.

INTRODUCTION

Wild and cultivated blackberries have been used as food and medicine for hundreds of years (Mazza and Miniati, 1993), yet the analysis of blackberry flavors has been limited compared to other small fruits such as raspberry and strawberry (Honkanen and Hirvi, 1990; Shamaila et al., 1993; Zabetakis and Holden, 1997; De Ancos et al., 2000; Hakala et al., 2002). Independent analyses were made on anthocyanins (Mazza and Miniati, 1993), sugars and acids (Wrolstad et al., 1980; Plowman, 1991), and volatile and bound volatile compounds in fresh or processed blackberries (Scanlan et al., 1970; Houchen et al., 1972; Gulan et al., 1973; Georgilopoulos and Gallois, 1987a, 1987b, 1988; Humpf and Schreier, 1991; Herrmann, 1992; Li et al., 1998). Six of the volatile studies (1970, 1972, 1973, 1987a, 1987b, 1988) analyzed 'Evergreen' cultivars.

Although many volatiles (147) are reported in fresh blackberries (Nijssen et al., 1996), few compounds were specifically described as "blackberry-like". Major fresh 'Evergreen' volatiles include 2-heptanol (43.06%), *p*-cymen-8-ol (3.72%), 2-heptanone (3.32%), 1-hexanol (3.05%), α-terpineol (2.38%), pulegone (2.05%), 1-octanol (1.83%), isoborneol (1.76%), myrtenol (1.28%), 4-terpineol (1.21%), carvone (1.14%), elemicine (1.12%), and 1-nonanal (1.01%) (Honkanen and Hirvi 1990). Twenty-two volatiles were identified in processed 'Evergreen' blackberry essence (Scanlan et al., 1970; Houchen et al., 1972; Gulan et al., 1973).

Prior to the early 1980's, the most widely planted blackberry cultivar in the world was the 'Thornless Evergreen' (*Rubus laciniatus* Willd.). Since that time the 'Marion' blackberry (*Rubus sp.* L.) has become the predominant cultivar planted (Strik, 1992; Finn et al., 1997). Consumers greatly prefer 'Marion' blackberry flavor (Strik, 1992; Finn et al., 1997); this has stimulated

blackberry research to correlate quantifiable flavor characteristics to berry genetic makeup, as part of breeding efforts to develop new thornless, winter-hardy blackberry cultivars with 'Marion' flavor. Previous blackberry research focused on chemical identification of compounds; they did not examine the aroma contribution of these compounds to blackberry flavor. Little aroma research has been done on the 'Thornless Evergreen' blackberry, none has been done on the 'Marion', and none made to examine cultivar differences.

Although the differences between 'Marion' and 'Thornless Evergreen' blackberries have been subjectively described (Finn et al., 1997), no rigorous aroma comparison studies have been done. This study isolated, identified, and compared the most significant aromas of 'Marion' and 'Thornless Evergreen' blackberries via dynamic headspace sampling (purge-and-trap) and gas chromatography-olfactometry (OSME)/mass spectrometry (GC-O/MS).

MATERIALS & METHODS

Materials

'Marion' and 'Thornless Evergreen' blackberries, donated by an Oregon producer, were grown in Woodburn, Oregon from 5-10 year-old plants. The cultivars were machine and hand-harvested, washed, graded, individually quick frozen (IQF), and stored at –18 °C. One box of each cultivar (13.6 kg, frozen 5 months) was transported on ice to the laboratory, where they were stored at –23°C. Samples had been frozen for 8 months when analyzed. Two-octenal was obtained from Compagnie Parento Inc. (Toronto, Ontario, Canada); butyl acetate, 2-heptanone, 2-nonanone, α- and β-pinene, α-terpineol, and 2-undecanone were obtained from K & K Laboratories (Jamaica, New York, U.S.A.); methylhexanoate, octanol, pentanal, and 2-pentanol were obtained from Eastman (Rochester, New York, U.S.A.); benzyl acetate,

ethylhexanoate, hexyl acetate, linalool, and octanal were obtained from Aldrich Chemical Co. Inc. (Milwaukee, Wi., U.S.A.); diacetyl and methional were obtained from Sigma (St. Louis, Mo., U.S.A.). Sodium chloride and calcium chloride was obtained from Fisher Scientific (Fair Lawn, New Jersey, U.S.A.).

Sample Preparation

Three hundred grams of IQF berries were thawed at room temperature in a single layer for 3 hrs. The berries were combined with 30 g of NaCl, 3 g of $CaCl_2$, and 100 mL of distilled water in a glass blender jar (Waring products Division, Dynamics Corp. of America, New Hartford, Conn., U.S.A.) and blended by pulsing for a total of 3 min at high speed. Calcium chloride was added to inhibit enzyme activity as described by Buttery and others (1987). The puréed fruit was then transferred to a ground glass stoppered flask, stored in the refrigerator, and used immediately.

Dynamic headspace (Purge-and-trap) volatile isolation

Tekmar ALS 2016 and LSC 2000 purge-and-trap equipment (Tekmar Co., Cincinnati, Oh., U.S.A.) was used for dynamic headspace sampling of volatile compounds. A small plug of silane treated glass wool (Alltech Associates Inc., Deerfield, Ill., U.S.A.) was used to cover the frit of a 25 mL fritted glass sparger, and 12.8 g of blackberry puree was introduced. The sample was preheated for 5 min at 50 °C, and volatiles purged from the continuously heated sparger by a nitrogen gas flow of 40 mL/min for 40 min. Volatiles were adsorbed by a Tenax® trap (#12-0083-003, Tekmar Co.), and after the purge process, the trap was dry purged with nitrogen for 3 min.

Volatiles were then thermally desorbed (250 °C for 2 min) and transferred with helium carrier gas directly to the GC injection port by a 1.5 m x 1.6 mm i.d. transfer line. Headspace sampling was controlled by Teklink v. 3.0B software (Tekmar Co.).

Capillary gas chromatography-mass spectrometry

Capillary gas chromatography-mass spectrometry (GC-MS) analysis was performed with an Agilent 6890 gas chromatograph equipped with an Agilent 5973 Mass Selective Detector (MSD). System software control and data management/analysis was performed through Enhanced ChemStation Software, G1701CA v. C.00.01.08 (Agilent Technologies, Inc., Wilmington, De., U.S.A.). Volatile separation was achieved with two fused silica capillary columns: a 30 m x 0.32 mm i.d. column, coated with cross-linked 5% phenyl-methyl polysiloxane, film thickness 1 μm (DB-5; J&W Scientific, Folsom, Calif., U.S.A.), and a 30 m x 0.25 mm i.d. column, coated with cross-linked polyethylene glycol 20M, film thickness 0.5 μm (DB-Wax; J&W Scientific). The oven temperature was programmed for a 2 min hold at 40 °C, then 40 to 100 °C at 2 °C/min, then 100 to 230 °C at 10 °C/min (5 min hold). Injector and detector transfer line temperatures were 250 and 280 °C, respectively. The helium column flow rate was 2.0 mL/min, and the injections were splitless. Retention indices were estimated in accordance with a modified Kovats method (Van den Dool and Kratz, 1963). Electron impact mass spectrometric data were collected at an ionization voltage of 70 eV and an ion source temperature of 230 °C. Mass spectra of unknown volatiles were compared with those in the Wiley 275.L (G1035) Database (Agilent).

Gas chromatography-olfactometry

Separated blackberry volatiles were evaluated for sensory impact using the gas chromatography-olfactometry (GC-O) method known as OSME (Da Silva and others, 1994). Using the columns and chromatographic conditions described for the GC-MS analysis, column effluent was split 1:1 to the MSD and a sniffing port via a fused silica outlet splitter (Alltech Associates Inc.). The sniffing port was wrapped and heated by electrical heating tape (1.3 cm x 61 cm, Fisher Scientific), and the port effluent was mixed with humidified air. Six student volunteers at the Dept. of Food Science and Technology performed OSME evaluation of volatiles. Each subject performed 2 sessions of GC-O sniffing runs. A session consisted of two 50 min runs on a given column, 1 run for each blackberry cultivar, with a 20 min break between runs. For each volatile detected, subjects recorded a retention time, sensory description, and sensory impact. Subjects also marked the onset and endpoint of a perceived odor by means of an electrical push-button; these signals were stored with the sample MS data for later analysis. Subjects were free to choose any linear sensory impact scale; their ratings were normalized to a 15-point scale, where 1 meant a volatile had slight sensory impact (barely detected), 3 slight, 7 moderate, 11 large, and 15 extreme. For analysis and reporting purposes, a volatile was considered to have sensory impact if 3 or more subjects detected it, and its average normalized impact was 5 or greater.

RESULTS & DISCUSSION

Tables 2.1 and 2.2 list 'Marion' and 'Thornless Evergreen' blackberry volatiles separated with polar and non-polar columns. Combined table data shows a total of 30 peaks common to both cultivars; 10 aldehydes, 5 esters, 6 ketones, 3 alcohols, 3 hydrocarbons (terpenes), allo-ocimene, and Theaspiranes A and B. 'Marion' volatiles also include 6 additional esters, 1 ketone, 1 alcohol, 3 hydrocarbons (terpenes), neo allo-ocimene, and dimethyltrisulfide. 'Thornless Evergreen' volatiles additionally include 2 aldehydes, 1 ester, 1 ketone, 6 alcohols, 2 hydrocarbons (terpenes), 2 phenols, and t-β-ocimene. Not listed in the tables were a total of 38 unknown aroma peaks detected by panelists. These unknowns were generally described as fruity (5), floral (3), fruity/floral (10), chemical/fermented (14), and vegetal (6). Possibly these unknown aromas did not provide identifying mass spectral data due to low concentrations of compounds and/or co-elution within prominent peaks of identified volatiles. Diacetyl (2,3-butanedione) was tentatively reported by Georgilopoulos and Gallois (1987a); this study confirms its presence in both cultivars. Georgilopoulos and Gallois (1987a) also identified Theaspiranes A and B in Evergreen, but reported they had no odor intensity; this study reports the Theaspiranes have moderate odor intensities. These differences are probably due to the different extraction methods used.

Table 2.1: Blackberry compounds separated with DB-Wax column

Wax RI	Intensity[a] 'Marion'	Intensity[a] 'Evergreen'	Compound[b]	Aroma descriptors this study, (literature)	Basis[c] of Identification
727	8	6	Acetaldehyde* [r]	alcohol, acetaldehyde, (pungent)	A, MS, RI
982	12	11	2,3-Butanedione* [r]	butter, (butter)	A, MS, RI
1023	8	9	α–Pinene [r]	piney, earthy, perfume, (pine, turpentine)	A, MS, RI
1052	10	10	Ethyl 2-methylbutanoate [r]	berries, fruity, pineapple, (fruity, pineapple)	A, MS, RI
1091	7	8	Hexanal [r]	grassy,green, (grassy, green, fruity on dil)	A, MS, RI
1188	8	6	2-Heptanone [r]	berry, fruity, sweet, floral, (spicy, banana, fruity)	A, MS, RI
1194	10	10	Methyl hexanoate [r]	fruity, sweet, pineapple, (pineapple, ethereal)	A, MS, RI
1240	7	6	Ethyl hexanoate [r]	fresh, floral, blackberry, (fruity, powerful)	A, MS, RI
1305	9	10	1-Octen-3-one* [r]	crayon, mushroom, metallic, (mushroom, metallic)	A, RIL, T
1335	11	9	2-Heptanol [r]	spicy piney, floral, harsh, (brassy herbaceous, earthy, oily)	A, MS, RI
1399	10	7	Nonanal [r]	floral, rose, fruity, (floral, citrus, on dil orange, rose)	A, MS, RI
1462	8	7	Methional*	baked potato, earthy, unpleasant, (onion, meaty, earthy)	A, RI
1499	5	6	Theaspirane A* [r]	earthy, fragrant, geranium, (woody, camphoraceous)	A, MS, RIL
1548	8	7	Theaspirane B* [r]	dusty, woody, floral, (woody, ionone-like, fruity)	A, MS, RIL
1563	11	8	Linalool [r]	minty, floral, citrus, (light, floral, citrus)	A, MS, RI
1710	9	10	α–Terpineol [r]	floral, over-ripe fruit, perfume, (sweet lilac, citrus, pine oil)	A, MS, RI
1837	10	8	β–Damascenone [r]	floral, sweet, blackberry, (sweet, fruity, exotic floral)	A, MS, RIL
921	8		3-Methylbutanal	malty, mint, (malty, herbaceous when dil)	A, MS, RI
1014	8		Methyl 2-methylbutanoate*	fruity,floral,sweet, (fruity, sweet)	A, MS
1041	8		Ethyl butanoate	fruity, artificial fruit, (fruity, sweet, ethereal)	A, MS, RI
1075	9		Butyl acetate	fruity, floral, artificial berry, (fruity, diffusive)	A, MS, RI
1107	7		2-Methylpropanol* [r]	waxy, chemical, mint, (disagreeable, sweet-musty)	A, MS, RI
1169	8		β–Myrcene* [r]	grassy, metallic, geranium, (pleasant, balsamic, metallic)	A, MS, RIL

Table 2.1 (continued): Blackberry compounds separated with DB-Wax column

Wax RI	Intensity[a] 'Marion'	Intensity[a] 'Evergreen'	Compound[b]	Aroma descriptors this study, (literature)	Basis[c] of Identification
1377	11		Dimethyltrisulfide*	sulfury, rancid cheese, wet rag, rotten, (alliaceous, penetrating)	A, MS, RI
1395	9		2-Nonanone* [r]	green, floral, mint, (characteristic rue/evergreen)	A, MS, RI
1587	10		Nonyl acetate*	cucumber, rose, fresh grass, (fruity, soapy, gardenia)	A, MS
1740	10		Benzyl acetate* [r]	sweet candy, red licorice, spicy, (jasmine, fruity, fresh)	A, MS, RI
1221		8	3-Methylbutanol [r]	dried fish, malty, moldy, (fusel oil, pungent, whisky)	A, MS, RI
1227		7	t-2-Hexenal [r]	organic, fruity, (fruity, vegetable, green, sweet)	A, MS, RIL
1256		7	t-β-Ocimene*	metal, baked, (warm, herbaceous)	MS, RIL
1293		5	Octanal	organic, citrus, lemon, (fatty, citrus)	A, MS, RI
1377		10	allo-Ocimene*	earthy, cooked fruit, green, (warm, herbaceous)	MS, RIL
1397		5	cis-3-Hexenol [r]	green piney, grassy, (green, on dil herbaceous leafy/fresh)	A, MS, RIL
1410		5	1-Octen-3-ol	mushroom, (mushroom)	A, RIL, T
1576		10	Octanol [r]	organic, citrus, (fatty, citrus, orange-rose)	A, MS, RI
1751		11	*l*-Carvone	peppermint, licorice, mint, (peppermint, warm, herbaceous)	A, MS
1794		7	*p*-Methyl-acetophenone	fruity, sweet, floral, (sweet/strong fruity, floral)	A, MS

a 15 point scale. 1 = just detected, 15 = extreme impact.

b Asterisk means not previously reported in blackberry, r = reported in red raspberry.

c A = study aromas synonymous with literature, MS = mass spectral data, RIL = retention index from literature, RI = retention index from standards, T = tentative identification.

Table 2.2: Blackberry compounds separated with DB-5 column

DB5 RI	Intensity[a] 'Marion'	Intensity[a] 'Evergreen'	Compound[b]	Aroma descriptors this study, (literature)	Basis[c] for Identification
<500	6	6	Acetaldehyde* [r]	alcohol, vinegar, (pungent)	A, MS, RI
551	7	7	2-Methylpropanal*	chemical, unpleas, earthy, (malty, pungent)	A, MS, RI
584	11	10	2,3-Butanedione* [r]	butter, (butter)	A, MS, RI
680	11	10	1-Penten-3-one*	plastic, latex, ink, gasoline, (chemical, pungent, spicy)	A, MS, RI
694	7	6	Pentanal	butter, floral, (pungent, when dil nutty, warm)	A, MS, RI
795	9	10	Hexanal [r]	berry, green, grassy, (grassy, green, fruity on dil)	A, MS, RI
846	10	10	t-2-Hexenal [r]	berry, sweet, pineapple, (fruity, green, sweet, fragrant)	A, MS, RIL
953	10	10	Benzaldehyde [r]	fruity, berry, raspberry, (bitter almond, sweet, fragrant)	A, MS, RIL
968	10	9	1-Octen-3-one* [r]	mushroom, (mushroom, metallic)	A, MS, RIL
975	9	11	β-Pinene [r]	geranium, woody, vegetative, (dry, woody, turpentine)	A, MS, RI
992	8	8	Ethyl hexanoate [r]	sweet, citrus, floral, blackberry, (fruity, powerful)	A, MS, RI
1005	8	7	Hexyl acetate [r]	banana, floral, fruity, ethereal, (floral, fruity, pear)	A, MS, RI
1060	6	7	2-Octenal	caramel, organic, coffee, (green, herbaceous, cognac, honey)	A, MS, RI
1090	7	7	α-Terpinolene [r]	earthy, chemical, musty, (plastic, petroleum, sweet pine)	A, MS
1094	11	9	Linalool [r]	plastic, floral, berry, moldy wine, (light, floral, citrus)	A, MS, RI
1098	10	9	Nonanal [r]	floral, orange, citrus, (floral, citrus, orange, rose)	A, MS, RI
1292	8	8	2-Undecanone [r]	pine, tea, floral, musty, soap, (citrus, rose, iris, rue)	A, MS, RI
1384	8	6	Hexyl hexanoate*	plum, bell pepper, fruity, (fresh vegetable, fruity)	A, MS
1401	7	8	β-Damascenone [r]	juicy bramble, spice, cooked berry, (sweet, fruity, exotic floral)	A, MS, RIL
798	6		Ethyl butanoate	berry, strawberry, floral, (fruity, sweet, ethereal)	A, MS, RI
908	12		2-Heptanol [r]	dried fish, earthy, spicy, (brassy herbaceous, earthy, oily)	A, MS, RI
964	8		Dimethyltrisulfide*	unpleasant, oniony, earthy, (alliaceous, penetrating, meaty)	A, MS, RI
1000	7		α-Phellandrene [r]	tomato, grassy, (minty, herbaceous)	A, MS

Table 2.2 (continued): Blackberry compounds separated with DB-5 column

DB5 RI	Intensity[a] 'Marion'	Intensity[a] 'Evergreen'	Compound[b]	Aroma descriptors this study, (literature)	Basis[c] for Identification
1065	5		γ-Terpinene [r]	spicy, floral, perfume, (herbal, citrus)	A, MS
1128	9		allo-Ocimene*	fermented rice, spicy,plastic, (warm, herbaceous, citrus)	A, MS
1146	7		neo allo-Ocimene*	vegetal, melon, cucumber (warm, herbaceous, citrus)	MS, RIL
1257	6		2-Phenylethylacetate*	orange, raspberry, sweet, (sweet, fruity, rose)	A, MS, RIL
646		10	3-Methylbutanal	plastic, chemical, yeasty, (malty, herbaceous when dil)	MS, RIL
897		11	Methional*	baked potato, herbal, (onion, meaty, earthy)	A, RI
933		6	α-Pinene [r]	pine, fir/evergreen, (pine, turpentine)	A, MS, RI
942		7	Camphene [r]	burnt rubber, unpleasant, mint, (dull, camphoraceous)	A, MS
1110		7	Octyl formate*	earthy, citrus, perfume, (orange, fruity, rose)	A, MS
1156		9	t-2-nonenal*	vegetative, green fruit, fatty, wood, (fatty, waxy, cucumber)	A, MS, RIL
1181		6	4-Terpineol [r]	mold, rotten citrus, (musty, dusty)	A, MS
1218		9	Myrtenol [r]	bread, hot cereal, pungent, (minty, medicinal, woody, camphor)	A, MS
1249		6	*l*-Carvone	anise, mint, fruit, (warm, herbaceous, peppermint)	A, MS
1360		6	Eugenol [r]	benzene, wood, dirt, (cinammon, clove)	A, MS
>1500		5	Elemicin	floral, sweet spicy, (weak spicy, woody, floral)	A, MS

a 15 point scale. 1 = just detected, 15 = extreme impact.

b Asterisk means not previously reported in blackberry; r = reported in red raspberry.

c A = study aromas synonymous with literature, MS = mass spectral data, RIL = retention index from literature, RI = retention index from standards, T = tentative identification.

Panelists did not rate any aroma intensity as extreme (intensity = 15); 68% of aroma intensities were rated low-moderate to moderate (5-8), 32% were rated moderately-large to large (9-12), and only 9% of aroma intensities were rated large (11-12). Results support observations that odor specific sensitivities of GC-O frequently exceed that of GC/MS (Acree, 1997), and demonstrate the viability of GC-O as a preliminary qualitative and quantitative aroma analysis tool, notwithstanding concerns for method cost and statistical noise from evaluation variability between and within panelists (Acree, 1997; Guichard et al., 1995; Pollien et al., 1997).

Disregarding unknowns, data indicates essentially equal numbers of the compound classes mentioned, except for alcohols ('Thornless Evergreen' = 2.25 x 'Marion'), esters ('Marion' = 1.8 x 'Thornless Evergreen'), and some miscellaneous compounds (dimethyltrisulfide, eugenol, elemicin, ocimene isomers). Primary aroma descriptors for blackberry volatiles are chemical, earthy, floral, fruity, green, herbaceous, spicy, and vegetal, yet of 58 reported compounds, only 7 prompted panelist aroma descriptors specific to bramble fruit, that is, berry, blackberry, bramble, or raspberry. Thirty-five of the 58 compounds have been reported in red raspberry; 22 of the 58 have not been previously reported in blackberry fruit (Burdock, 1995; Georgilopoulos and Gallois, 1987a; Nijssen et al., 1996; Roberts and Acree, 1996). Nine compounds of the 22 were reported in red raspberry; 5 (acetaldehyde, diacetyl, 1-octen-3-one, Theaspiranes A and B) were found in both 'Marion' and 'Thornless Evergreen', 4 (benzyl acetate, β-myrcene, 2-methylpropanol, 2-nonanone) only in 'Marion'. Identification of some of these newly reported volatiles is probably due to the extraction and analysis methods used, while others reflect the 'Marion' blackberry's pedigree, which contains at least five *Rubus* species, including raspberry (Finn et al., 1997). Raspberry aroma contains 1 compound out of 213 (4-(*p*-hydroxyphenyl)-2-butanone, the

"raspberry ketone") specifically described as "characteristic raspberry taste and odor" (Burdock, 1995; Njissen et al., 1996). In contrast, characteristic blackberry aroma is apparently the result of a more complex formulation of volatiles.

CONCLUSION

The GC-O/MS method is a viable procedure to isolate and identify odor-active volatiles in blackberry fruit. The aroma profiles of 'Marion' and 'Thornless Evergreen' blackberries are complex, and further study (solvent extraction, aroma extract dilution analysis (AEDA), odor activity values (OAV), aroma compound quantification) is required to clarify their specific compositions and characterize cultivar differences.

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CHAPTER 3.

AROMA EXTRACT DILUTION ANALYSIS OF 'MARION' (*Rubus sp.* L.) AND 'THORNLESS EVERGREEN' (*R. laciniatus* L.) BLACKBERRIES

KEITH KLESK AND MICHAEL QIAN

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ABSTRACT

'Marion' and 'Thornless Evergreen' blackberry aromas were analyzed by aroma extract dilution analysis (AEDA). Sixty-three aromas were identified (some tentatively) by mass spectrometry (MS) and gas chromatography-retention time (GC-RT); 48 were common to both cultivars, and 27 have not been previously reported in blackberry fruit. Comparison of cultivars shows both have comparable compound types and numbers, but with widely differing aroma impacts, as measured by flavor dilution (FD) factors. Ethyl 2-methylbutanoate, ethyl 2-methylpropanoate, hexanal, furanones (2,5-dimethyl-4-hydroxy-3(2H)-furanone, 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone, 4-hydroxy-5-methyl-3(2H)-furanone, 4,5-dimethyl-3-hydroxy-2(5H)-furanone, 5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone) and sulfur compounds (thiophene, dimethylsulfide, dimethyldisulfide, dimethyltrisulfide, 2-methylthiophene, methional) were prominent in 'Thornless Evergreen' (FD 512 to 2048). Except for ethyl 2-methylpropanoate, these same compounds were also prominent in 'Marion', but the FD factors varied significantly (FD 8 to 256) from 'Thornless Evergreen'. The aroma profile of blackberry is complex, as no single volatile was unanimously described as characteristically blackberry.

INTRODUCTION

Blackberries are popular fruits because of their flavor and nutritional content; they have been used as food and medicine for hundreds of years (Mazza and Miniati, 1993). Used fresh and processed into a variety of food products, blackberries are extensively cultivated, yet their aroma compositions have not been detailed compared to the aromas of other small fruits such as raspberry and strawberry (Honkanen and Hirvi, 1990; Shamaila et al., 1993; Zabetakis and Holden, 1997; De Ancos et al., 2000; Hakala et al., 2002). Independent analyses of blackberries examined anthocyanins (Mazza and Miniati, 1993), sugars and acids (Wrolstad et al., 1980; Plowman, 1991), and volatile and bound volatile compounds in fresh or processed blackberries (Scanlan et al., 1970; Houchen et al., 1972; Gulan et al., 1973; Georgilopoulos and Gallois, 1987a, 1987b, 1988; Humpf and Schreier, 1991; Herrmann, 1992; Li et al., 1998). Most of the studies investigated volatile compounds (Scanlan et al., 1970; Houchen et al., 1972; Gulan et al., 1973; Georgilopoulos and Gallois, 1987a, 1987b, 1988) in 'Evergreen' and 'Thornless Evergreen'. A total of 147 volatiles have been reported in fresh blackberries (Nijssen et al., 1996), but very few studies were about aroma-active compounds, and few compounds were specifically described as "blackberry-like".

The 'Marion' blackberry (*Rubus sp.* L.) has a distinctive flavor greatly preferred by consumers; consequently it has replaced the 'Thornless Evergreen' (*Rubus laciniatus* Willd.) as the predominant cultivar planted in the Pacific Northwest (Strik, 1992; Finn et al., 1997). Consumer preference for the 'Marion' has stimulated research to correlate quantifiable blackberry flavor characteristics to berry genetic makeup, in order to breed new thornless blackberry cultivars with 'Marion' flavor. Since the aroma differences

between 'Marion' and 'Thornless Evergreen' have been only subjectively described (Finn et al., 1997), the purpose of this investigation was to identify, rank, and compare the odor-active compounds in the two cultivars using aroma extract dilution analysis (AEDA) and gas chromatography-mass spectrometry (GC-MS).

MATERIALS & METHODS

Chemicals

Authentic aroma standards were obtained as follows: butyl acetate, limonene, octyl acetate, octyl formate, 2-heptanone, and 2-undecanone (K&K Laboratories, Jamaica, NY). Methyl hexanoate and octanol (Eastman, Rochester, NY). Acetaldehyde, acetic acid, β-ionone, butanoic acid, *l*-carvone, 2,5-dimethyl-4-hydroxy-3-(2H)-furanone, dimethyldisulfide, dimethyltrisulfide, ethyl acetate, ethyl butanonate, ethyl hexanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl 2-methylpropanonate, eugenol, 2-heptanol, hexanal, hexanoic acid, *t*-2-hexenal, linalool, p-methylacetophenone, 3-methylbutanal, methyl butanoate, 2-methylbutanoic acid, nonanal, *t*-2-nonenal, octanol, 1-octen-3-ol, 1-octen-3-one, phenethyl alcohol (Aldrich Chemical Co. Inc., Milwaukee, WI). Diacetyl and methional (Sigma Chemical Co., St. Louis, MO).

Blackberry Samples

'Marion' and 'Thornless Evergreen' blackberries, donated by an Oregon producer, were grown in Woodburn, Oregon from 5-10 year-old plants. The fruits (both machine and hand-harvested), were washed, graded,

individually quick frozen (IQF), and stored at –18 °C. One box of each cultivar (13.6 kg, frozen 5 months) was transported on ice to the laboratory, where they were stored at –23°C. Samples had been frozen for nine months when analyzed.

Extraction of Volatile Compounds

For each cultivar, one kilogram of IQF blackberries was thawed at room temperature in a single layer for 3 hrs. The berries were combined with 100 g of NaCl, and 10 g of $CaCl_2$ in a commercial blender and blended by pulsing for a total of 3 min at high speed. Calcium chloride was added to inhibit enzyme activity as described by Buttery and others (Buttery et al., 1987). The puréed fruit was passed through a commercial stainless steel food mill to remove seeds. The seed pulp was batch extracted 3 times with freshly distilled pentane:diethyl ether (1:1 v/v) while the seedless purée was extracted 3 times in a separatory funnel. The extracts were combined to yield a total volume of 880 mL. Non-volatiles were removed from the organic extract using solvent assisted flavor extraction (SAFE) at 50°C under vacuum according to the method proposed by Engel and others (Engel et al., 1999). The organic SAFE extract was dried with anhydrous Na_2SO_4, concentrated to 1 mL by solvent distillation, and reduced to its final volume of 0.1 mL with a flow of nitrogen.

GC/O Analysis

The analysis was performed using a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector (FID) and an olfactometer. Samples were analyzed on a Stabilwax column (30 m x 0.32 mm i.d. cross-linked polyethylene glycol, 1 μm film thickness, Restek Corp., Bellefonte, PA), and a DB-5 column (30 m x 0.32 mm i.d., cross-linked

phenyl-methyl polysiloxane, 1μm film thickness, J&W Scientific, Folsom, CA). Column effluent was split 1:1 (by volume) into the FID and a heated sniffing port with a fused silica outlet splitter (Alltech Associates, Inc., Deerfield, IL). Injector and detector temperatures were 250 °C. The helium column flow rate was 2.0 mL/min, and the 2 μL sample injections were splitless. The oven temperature was programmed for a 2 min hold at 40 °C, then 40 to 100 °C at 5 °C/min, then 100 to 230 °C at 4 °C/min (10 min hold). Retention indices were estimated in accordance with a modified Kovats method (Van den Dool and Kratz, 1963).

AEDA

Flavor dilution (FD) factors for the odor-active compounds in each cultivar were determined using AEDA (Schieberle and Grosch, 1987). Concentrated samples were serially diluted with 1:1 (v/v) pentane:diethyl ether (1+1). GC/O with two experienced panelists was then performed with 2 μL injections of original samples and diluted extracts.

GC-MS Analysis

Analysis of the original concentrated AEDA samples was performed using an Agilent 6890 gas chromatograph equipped with an Agilent 5973 Mass Selective Detector (MSD). System software control and data management/analysis was performed through Enhanced ChemStation Software, G1701CA v. C.00.01.08 (Agilent Technologies, Inc., Wilmington, DE). Volatile separation was achieved with two fused silica capillary columns: a 30 m x 0.32 mm i.d. Stabilwax (cross-linked polyethylene glycol) column with a 1 μm film thickness (Restek, Bellefonte, PA), and the other a 30 m x 0.25 mm i.d. DB-5 (cross-linked phenyl-methyl polysiloxane) column with a 0.25 μm film thickness (J&W Scientific, Folsom, CA). The helium

column flow rate was 2.0 mL/min, and the 2 μL sample injections were splitless. The oven temperature was programmed as for the GC/O analysis. Injector, detector transfer line, and ion source temperatures were 250, 280, and 230 °C, respectively. Electron impact mass spectrometric data from m/z 35-300 was collected at 5.27 scans/s, at an ionization voltage of 70 eV. Retention indices were estimated in accordance with a modified Kovats method (Van den Dool and Kratz, 1963). Compound identifications were made by comparing aromas with authentic standards and Kovats retention indices (RI), RI reported in literature (J. Agric. Food Chem., Rychlik et al., 1998, among others), and/or mass spectral data from the Wiley 275.L (G1035) Database (Agilent Technologies, Inc., Wilmington, DE).

Further identification of some aroma compounds by GC-MS Analysis.

To further clarify AEDA volatile composition, for each cultivar, five kilograms of IQF blackberries were thawed at room temperature in a single layer for 3 hrs. The berries were blended by pulsing for a total of 3 min at high speed in a commercial blender, and the purée poured into a stainless steel pan. Concentrated pectolytic enzyme (Vinozym® FCE G, Novo Nordisk, Franklinton, NC) was prepared and thoroughly mixed into the purée. A total of 0.15 g enzyme was added to the 'Marion' purée, and 1.0 g added to the more viscous 'Thornless Evergreen' purée. The mixture was covered with aluminum foil and left to stand at room temperature overnight (15 hours). Five hundred grams of NaCl was blended in, and the mixture strained and extracted as for the GC/O analysis using CH_2Cl_2 (total volume 2400 mL). The extraction produced an emulsion that was broken with centrifugation for 20 minutes (1800 rpm, approx. 1000 g). The organic extract was then further prepared as for the GC/O analysis, and reduced to its final volume of 0.2 mL

with a flow of nitrogen. Analysis conditions and methods were identical to those used for the 1 kilogram samples, except that 5 μL of sample were injected, and the oven temperature programmed for a 2 min hold at 40 °C, then 40 to 230 °C at 1°C/min (2 min hold).

RESULTS & DISCUSSION

Tables 3.1 and 3.2 list 'Marion' and 'Thornless Evergreen' blackberry volatiles separated with polar and non-polar columns. On the polar column, a total of 51 aroma compounds were detected, with 45 of them identified. On the non-polar column, 55 aromas were detected, and 51 of them were identified. Among these identified aromas, 12 were detected on the polar column only, while 17 were detected on the non-polar column only. Combined data (Table 3.3) shows 63 odor-active volatiles were detected, and 48 were common to both cultivars. 'Marion' contained 60 of 63 volatiles, and 'Thornless Evergreen' 51.

The most significant (FD≥16) odor-active volatiles in 'Marion' determined on the non-polar (DB-5) column were methional (FD = 256); ethyl 2-methylbutanoate (FD = 128); benzaldehyde and hexanal (FD = 64); 2-methylbutanoic acid, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, 4,5-dimethyl-3-hydroxy-2(5*H*)-furanone, ethyl hexanoate, dimethyldisulfide, and 2-methylthiophene (FD = 32); linalool, neo-allo-ocimene, dimethylsulfide, dimethyltrisulfide, and methylethylsulfide (FD = 16). In addition, 2-ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone, 4-hydroxy-5-methyl-3(2*H*)-furanone, and butanoic acid (FD = 32), ethyl acetate, acetic acid, and 2-heptanone (FD = 16) may also be important to 'Marion' blackberry flavor, as they had high flavor dilution factors as determined on the polar (Stabilwax) column.

Table 3.1: AEDA of 'Marion' and 'Thornless Evergreen' (Stabilwax column)

RI	Compound[a]	Aroma descriptors this study	Basis of identification[b]	FD Factor 'Marion'	'T. Evergreen'
811	Ethyl acetate[r]	floral, fruity	MS, RI	16	2
910	3-Methylbutanal	fresh grass, fruity, leaf	MS, RI	-	4
935	Dimethylsulfide[*] [(T)]	garlic bologna, cabbage	RIL	16	128
965	Ethyl 2-methylpropanoate[*]	sweet, fruity, berry, floral	RI	-	256
973	Methyl butanoate	fruity, sweet	RI	4	-
998	2,3-Butanedione (Diacetyl)[*r]	buttery	MS, RI	2	2
1030	Thiophene[*] [(T)]	garlic bologna, sulfury	RIL	8	128
1038	Ethyl butanoate	fruity, banana	MS, RI	4	-
1053	Ethyl 2-methylbutanoate[r]	fruity	MS, RI	4	-
1065	Ethyl 3-methylbutanoate	fruit, sweet, banana	RI	-	64
1082	Butyl acetate	fruity, juicy	MS, RI	2	-
1103	Dimethyldisulfide[*]	vegetal	MS, RI	2	-
1106	Hexanal[r]	green, fresh	MS, RI	-	4
1161	**Unk**	plastic, fatty, waxy		32	-
1191	2-Heptanone[r]	fruity, banana, sweet, floral	MS, RI	16	32
1202	Methyl hexanoate[r]	fruity, green, sweet	MS, RI	8	4
1251	Ethyl hexanoate[r]	fruity, floral	MS, RI	8	-
1326	1-Octen-3-one[*r]	mushroom, earthy	RI	8	16
1333	2-Heptanol[r]	woody, earthy, vegetal, minty	MS, RI	4	512
1369	Hexanol[r] [(T)]	floral, spice	MS, RIL	4	-
1383	Dimethyltrisulfide[*]	vegetal, garlic	MS, RI	2	16
1402	Nonanal[r]	floral, fruity	MS, RI	2	-

Table 3.1 (continued): AEDA of 'Marion' and 'Thornless Evergreen' ...

RI	Compound[a]	Aroma descriptors this study	Basis of identification[b]	FD Factor 'Marion'	'T. Evergreen'
1467	Acetic acid[r]	acid, sour	MS, RI	16	32
1476	1-Octen-3-ol	mushroom	RI	4	-
1490	Methional[*]	potato, earthy, onion	RI	32	512
1491	Octyl acetate	floral, sweet	MS, RI	4	-
1508	Theaspirane A[*r] (T)	floral, earthy, tea, green	MS, RIL	4	-
1550	Theaspirane B[*r] (T)	earthy, fruity, sweet	MS, RIL	4	-
1560	Linalool[r]	sweet, floral, berry, green	MS, RI	4	128
1574	Octanol[r]	waxy, fruity	MS, RI	2	-
1622	2-Undecanone[r]	floral, grn, pine, citrus	MS, RI	8	128
1634	**Unk**	roasted peanuts		2	-
1650	Butanoic acid[*r]	rancid cheese, sour, pungent	MS, RI	32	-
1693	2-Methylbutanoic acid[*r]	rancid cheese, sour, acid	MS, RI	32	128
1724	**Unk**	plastic curtain, waxy		-	32
1749	*l*-Carvone	peppermint, fresh leaf	MS, RI	8	4
1794	*p*-Methylacetophenone	fresh, green, floral, fruity	MS, RI	-	32
1851	β-Damascenone[r] (T)	sweet, floral, grape, blackberry	MS, RIL	4	32
1868	Hexanoic acid[*r]	pungent, sour	MS, RI	2	128

Table 3.1 (continued): AEDA of 'Marion' and 'Thornless Evergreen' ...

RI	Compound[a]	Aroma descriptors this study	Basis of identification[b]	FD Factor 'Marion'	'T. Evergreen'
1875	**Unk**	waxy citrus, lemon, woody		-	32
1905	Benzyl alcohol[r] (T)	sweet, citrus, grass	MS, RIL	2	2
1952	Phenethyl alcohol[r]	floral, perfume, peach	MS, RI	8	64
1967	**Unk**	grass, pungent, green, floral		-	8
2017	Cinnamic aldehyde (T)	sweet, spice, cinnamon	MS, RIL	4	-
2018	**Unk**	floral, grass, green		-	8
2053	2,5-dimethyl-4-hydroxy-3(2*H*)-furanone*[r]	fruity, sweet, caramel	RI	8	1024
2078	2-ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone* (T)	cooked bramble, sweet caramel	RIL	32	16
2114	4-hydroxy-5-methyl-3(2*H*)-furanone* (T)	caramel, strawberry, cooked bramble	RIL	32	8
2211	4,5-dimethyl-3-hydroxy-2(5*H*)-furanone* (T)	spice, curry, fruity	RIL	4	128
2246	5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone* (T)	roasted meat, cumin, maple syrup	RIL	4	256
2294	Cinnamic alcohol[r] (T)	floral, tea, sweet, fruity	RIL	8	32

a * = not previously reported in blackberry, r = reported in red raspberry, T = tentative identification

b MS = mass spectral data, RIL = retention index from literature, RI = retention index from standards.

Table 3.2: AEDA of 'Marion' and 'Thornless Evergreen' (DB5 column)

RI	Compound[a]	Aroma descriptors this study	Basis of identification[b]	FD Factor 'Marion'	'T. Evergreen'
<500	Acetaldehyde*r	grass, green	MS, RI	1	8
516	Dimethylsulfide* (T)	garlic, onion	RIL	16	8
557	2-Methylpropanal* (T)	wood, grass	MS, RIL	1	-
579	2,3-Butanedione (Diacetyl)*r	buttery	MS, RI	2	2
599	Acetic acidr	acetic acid, vinegar	RI	-	16
609	Methylethylsulfide* (T)	alliaceous, pungent	RIL	16	8
610	Ethyl acetater	fruity	RI	1	-
621	**Unk**	pungent		8	4
649	3-Methylbutanal	vegetal, earthy	MS, RI	1	1
661	Thiophene* (T)	sour, green, earthy, onion	RIL	4	2048
727	Dimethyldisulfide*	pungent, garlic, sulfury	MS, RI	32	2048
753	Ethyl 2-methylpropanoate*	fruity	RI	-	2048
758	2-Methylthiophene* (T)	earthy, pungent	RIL	32	512
770	Ethyl butanoate	fruity	MS, RI	2	-
791	Hexanalr	green, fresh	MS, RI	64	1024
808	Butanoic acid*r	cheesy, pungent	RI	1	2
848	Ethyl 2-methyl/3-methylbutanoater	fruity, sweet, berry, banana	RI	128	1024
854	2-Methylbutanoic acid*r (T)	cheesy, sour, smelly	RIL	32	16
874	t-2-Hexenalr	fruity, orange, green	MS, RI	1	4
897	Methional*	baked potato	RI	256	2048
906	2-Heptanolr	peppermint, green, woody	MS, RI	8	2
957	**Unk**	woody, floral, green		-	128

Table 3.2 (continued): AEDA of 'Marion' and 'Thornless Evergreen' ...

RI	Compound[a]	Aroma descriptors this study	Basis of identification[b]	FD Factor 'Marion'	'T. Evergreen'
959	Benzaldehyde[r] (T)	fruity, berry, juicy	MS, RIL	64	256
971	1-Octen-3-one*[r]	mushroom, earthy	MS, RI	2	16
979	Dimethyltrisulfide*	green veggie, garlic	RI	16	-
980	1-Octen-3-ol	woody, earthy, mushroom	RI	-	64
999	Hexyl acetate[r]	fruity	MS, RI	1	1
1002	Ethyl hexanoate[r]	floral, fruity	MS, RI	32	1
1033	Limonene[r]	overripe melon, green, tea	MS, RI	4	2
1042	t-β-Ocimene* (T)	sweet, floral, woody, perfume	RIL	8	2
1045	Benzyl alcohol[r] (T)	floral, fruity, rose	MS, RIL	1	2
1072	2,5-dimethyl-4-hydroxy-3(2*H*)-furanone*[r]	caramel, strawberry	RI	32	-
1087	4-hydroxy-5-methyl-3(2*H*)-furanone* (T)	cotton candy, sweet	RIL	4	-
1096	Linalool[r]	fruity, green, sweet, watermelon	MS, RI	16	8
1099	α-Terpinolene[r] (T)	woody, sweet, earthy	MS, RIL	1	64
1100	Nonanal[r]	watermelon, citrus, floral	MS, RI	8	8
1104	Octyl formate*	fruity	RI	1	-
1112	Phenethyl alcohol[r]	fruity, floral, rose, sweet	MS, RI	-	32
1131	4,5-dimethyl-3-hydroxy-2(5*H*)-furanone* (T)	roasted vegetables, sweet, caramel	RIL	32	4
1136	2-ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone* (T)	floral, sweet, caramel	RIL	2	2

Table 3.2 (continued): AEDA of 'Marion' and 'Thornless Evergreen' ...

RI	Compound[a]	Aroma descriptors this study	Basis of identification[b]	FD Factor 'Marion'	'T. Evergreen'
1149	neo-allo-Ocimene* (T)	citrus, vegetal, cucumber	MS, RIL	16	4
1161	t-2-Nonenal*	watermelon, fresh vegetable, green	MS, RI	1	64
1179	*p*-Methylacetophenone	floral, hot candy, sweet	RI	2	8
1234	*l*-Carvone	anise, fennel	RI	2	-
1255	5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone* (T)	caramel, smoky	RIL	1	-
1290	2-Undecanone[r]	wet grass, tea, floral, green	MS, RI	4	16
1305	Theaspirane A*[r] (T)	warm spices, vegetal, pungent	MS, RIL	2	1
1321	**Unk**	caramel, fruity, tea, green		-	16
1324	4-Vinylguaiacol*[r] (T)	BBQ rub, spicy	RIL	1	-
1370	Eugenol[r]	woody, citrus, spicy	RI	4	4
1392	β-Damascenone[r] (T)	floral, berry, sweet, grape	MS, RIL	8	4
1433	**Unk**	sweet, fruity, herbal, tea		2	-
1451	**Unk**	floral, spice, perfume, fruit, juicy		4	-
1496	β-Ionone[r]	floral, perfume, woody, spicy	MS, RI	2	16
1543	Elemicin (T)	green tea, spicy, perfume	RIL	1	-

a * = not previously reported in blackberry, r = reported in red raspberry, T = tentative identification

b MS = mass spectral data, RIL = retention index from literature, RI = retention index from standards.

Table 3.3: AEDA summary of 'Marion' and 'Thornless Evergreen'

Cultivar	Compound[a]	Cultivar	Compound[a]
	Acids		
Both	Acetic acid[r]	Both	Hexanoic acid[*r]
Both	Butanoic acid[*r]	Both	2-Methylbutanoic acid[*r]
	Alcohols		
Both	Benzyl alcohol[r]	Both	Linalool[r]
Both	Cinnamic alcohol[r]	'Marion'	Octanol[r]
'T. Evergreen'	Heptanol[r]	Both	1-Octen-3-ol
Both	2-Heptanol[r]	Both	Phenethyl alcohol[r]
'Marion'	Hexanol[r]		
	Aldehydes		
Both	Acetaldehyde[*r]	Both	Methional[*]
Both	Benzaldehyde[r]	Both	3-Methylbutanal
'Marion'	Cinnamic aldehyde	'Marion'	2-Methylpropanal[*]
Both	Hexanal[r]	Both	Nonanal[r]
Both	t-2-Hexenal[r]	Both	t-2-Nonenal[*]
	Esters		
'Marion'	Butyl acetate	'T. Evergreen'	Ethyl 2-methylpropanoate[*]
Both	Ethyl acetate[r]	Both	Hexyl acetate[r]
'Marion'	Ethyl butanoate	'Marion'	Methyl butanoate
Both	Ethyl hexanoate[r]	Both	Methyl hexanoate[r]
Both	Ethyl 2-methylbutanoate[r]	'Marion'	Octyl acetate
'T. Evergreen'	Ethyl 3-methylbutanoate	'Marion'	Octyl formate[*]

Table 3.3 (continued): AEDA summary of 'Marion' and 'Thornless Evergreen'

Cultivar	Compound[a]	Cultivar	Compound[a]
	Furanones		
Both	2,5-dimethyl-4-hydroxy-3(2*H*)-furanone*r	Both	5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone*
Both	4,5-dimethyl-3-hydroxy-2(5*H*)-furanone*	Both	4-hydroxy-5-methyl-3(2*H*)-furanone*
Both	2-ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone*		
	Hydrocarbons		
Both	Limonene[r]	Both	t-β-Ocimene*
Both	neo-allo-Ocimene*	Both	α-Terpinolene[r]
	Ketones		
Both	2,3-Butanedione (Diacetyl)*r	Both	β-Ionone[r]
Both	*l*-Carvone	Both	*p*-Methylacetophenone
Both	β-Damascenone[r]	Both	1-Octen-3-one*r
Both	2-Heptanone[r]	Both	2-Undecanone[r]
	Phenols		
'Marion'	Elemicin	'Marion'	4-Vinylguaiacol*r
Both	Eugenol[r]		
	Sulfur		
Both	Dimethyldisulfide*	Both	Methylethylsulfide*
Both	Dimethylsulfide*	Both	2-Methylthiophene*
Both	Dimethyltrisulfide*	Both	Thiophene*
	Theaspiranes		
Both	Theaspirane A*r	'Marion'	Theaspirane B*r

a Asterisk = not previously reported in blackberry, r = reported in red raspberry.

Many significant (FD≥16) odor-active volatiles in 'Thornless Evergreen' were identified on the DB-5 column. The most important aroma compounds included methional, ethyl 2-methylpropanoate, thiophene, and dimethyldisulfide (FD = 2048); hexanal and ethyl 2-methylbutanoate (FD = 1024); 2-methylthiophene (FD = 512); benzaldehyde (FD = 256); heptanol (FD = 128); 1-octen-3-ol, t-2-nonenal, and α-terpinolene (FD = 64); phenethyl alcohol (FD = 32); 1-octen-3-one, 2-undecanone, acetic acid, 2-methylbutanoic acid, and β-ionone (FD = 16). In addition, as determined on the polar (Stabilwax) column, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (FD = 1024); 2-heptanol (FD = 512); ethyl 2-methylpropanoate and 5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone (FD = 256); thiophene, linalool, 2-undecanone, hexanoic acid, 4,5-dimethyl-3-hydroxy-2(5*H*)-furanone and dimethylsulfide (FD = 128); ethyl 3-methylbutanonate (FD = 64); cinnamic alcohol, 2-heptanone, *p*-methylacetophenone, and β-damascenone (FD = 32); 2-ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone, 1-octen-3-one and dimethyltrisulfide (FD = 16) may also be important to 'Thornless Evergreen' blackberry flavor.

The cultivars have comparable compound types and numbers, but with widely differing aroma impacts, as measured by flavor dilution (FD) factors. Fresh 'Marion' blackberry aroma has been described as floral, fruity, sweet, caramel-fruity, and woody, while fresh 'Thornless Evergreen' aroma is spicy, green, herbaceous, fruity, and sweet. However, there are no prominent corresponding compositional differences between the cultivars within a volatile class. Both cultivars contain the same numbers of odor-active acids, furanones, hydrocarbons, ketones, and sulfur compounds. The 'Marion' contains 1 more theaspirane (Theaspirane B), 2 more alcohols (hexanol, octanol), aldehydes (cinnamic, 2-methylpropanal), and phenols (elemicin, 4-vinylquaiacol), and 5 more esters (methyl butanoate, ethyl butanoate, butyl

acetate, octyl acetate, octyl formate) than 'Thornless Evergreen'. The 'Thornless Evergreen' has 1 alcohol (heptanol) and 2 esters (ethyl 2-methylpropanoate, ethyl 3-methylbutanoate) not present in 'Marion'. Of 27 newly reported volatiles, 3 organic acids, 2 aldehydes, 5 furanones, 2 hydrocarbons, 2 ketones, 6 sulfur compounds, and 1 theaspirane are shared by the cultivars. This relatively large number of new volatiles is probably due to the extraction and analytical methods used. It is thought that some portion of 'Marion' aroma is due to its hybrid pedigree, which contains at least 5 *Rubus* species, including raspberry (Finn et al., 1997). However, although 35 volatiles in this study have been previously reported in red raspberry (Georgilopoulos and Gallois, 1987a; Fenaroli, 1995; Nijssen et al., 1996; Roberts and Acree, 1996), only 30 of them are common to both 'Marion' and 'Thornless Evergreen'; only 4 are unique to 'Marion'. Five volatiles out of 63 were described with aroma descriptors specific to *Rubus* fruit (berry, blackberry); no single compound was unanimously described as characteristically blackberry.

AEDA is a suitable method to screen potent odorants in blackberry, and results indicate that characteristic blackberry aroma is apparently a complex formulation of volatiles. 'Marion' and 'Thornless Evergreen' blackberries have many potent odorants in common, but qualitative aroma comparisons consistently note the more floral, caramel-fruity, sweet aroma of 'Marion' compared to the spicy, herbaceous, less fruity aroma of 'Thornless Evergreen'. Since a FD factor is the ratio of an odorant's concentration in an initial GC/O extract to its concentration in the most dilute extract that still allows detection, the value is a relative measure (Grosch, 1994), and does not conclusively determine that one cultivar contains more of a given aroma compound than another. Because the aroma profile of a food is, among others, a function of volatile concentrations and odor thresholds, the next step in identifying specific

aroma differences between 'Marion' and 'Thornless Evergreen' is the quantification of each aroma with a high FD factor, and calculation of its odor activity value (OAV), the ratio of the aroma concentration to its odor threshold in air. OAVs are better measures of which aroma compounds contribute to a cultivar's aroma, and of the differences in cultivar aroma profiles.

CONCLUSION

The GC-O/MS method of AEDA is a viable, suitable procedure for the initial screening of odor-active volatiles in blackberry fruit. The aroma profiles of 'Marion' and 'Thornless Evergreen' blackberries are complex, and apparently do not possess "character impact compounds" as raspberry does. Further study (aroma compound quantification and OAVs) is required to clarify their specific compositions and characterize cultivar differences.

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CHAPTER 4.

VOLATILE COMPOSITION OF 'MARION' (*Rubus sp.* L.) AND 'THORNLESS EVERGREEN' (*R. laciniatus* L.) BLACKBERRIES

ABSTRACT

'Marion' and 'Thornless Evergreen' blackberry volatiles were analyzed by capillary gas chromatography-flame ionization detection (GC-FID) and GC-mass spectrometry (GC-MS). One hundred and six volatiles were identified; 47 were common to both cultivars, and 46 have not been previously reported in blackberry fruit. Based on total percentage of FID area the 'Thornless Evergreen' contains significantly more alcohols, hydrocarbons, and phenols than the 'Marion'; the 'Marion' contains more acids and esters. Both cultivars contained comparable amounts of aldehydes and ketones, but alcohols were the most abundant. The six most abundant volatiles in 'Marion' were ethanol, acetic acid, hexanoic acid, ethyl acetate, linalool, and 2-heptanol; they totaled 52% of total peak area. In 'Thornless Evergreen' the six most abundant volatiles were 2-heptanol, ethanol, 2,3 butanediol, hexanol, α-pinene, and ethyl acetate; they totaled 43% of total peak area.

INTRODUCTION

Blackberries (genus *Rubus*, subgenus *Eubatus*) are a highly heterogenous, heteroploid, interfertile species. A versatile fruit, they are consumed fresh, but commercially most are processed into a variety of food products (Moore and Skirvin, 1990). Blackberries are found worldwide, but most domestication and commercial use of them has been made in North America (Moore and Skirvin, 1990). The Pacific Northwest of the United States extensively plants two economically important cultivars of trailing blackberry. The 'Thornless Evergreen' (*Rubus laciniatus* Willd.) was formerly the predominant cultivar planted, but in the early 1980's it was replaced by the 'Marion' (*Rubus sp.* L.), which is considered to have superior flavor (Strik, 1992; Finn et al., 1997).

The 'Thornless Evergreen' is a periclinal chimera of a selection of 'Evergreen' initially established in North America from Europe (Crandall, 1995; Bowling, 2000). Introduced in 1926, it is grown commercially only in the Pacific Northwest. The plants are vigorous and produce medium-sized, firm fruit (Moore and Skirvin, 1990; Crandall, 1995; Bowling, 2000) whose aroma has been described as spicy, green, herbaceous, fruity, and sweet (Klesk and Qian, 2003b). The 'Marion' blackberry is a hybrid introduced in 1956; its lineage is diverse and confusing. Its pedigree includes at least 5 *Rubus* species: *R. ursinus*, *R. armeniacus* Focke, *R. flagellaris* Willd., *R. aboriginum*, and *R. idaeus* L. (red raspberry) (Finn et al., 1997). Commercially grown only in the Pacific Northwest, the plants are very vigorous, and produce medium-large, medium-firm fruit (Moore and Skirvin, 1990) with an aroma described as floral, fruity, sweet, caramel-fruity, and woody (Klesk and Qian, 2003b).

Consumer preference for the 'Marion', along with consumer awareness of blackberry health and nutritional benefits (Moore and Skirvin, 1990; Poling, 1996) has stimulated efforts to breed new thornless blackberry cultivars with

'Marion' flavor. Recent studies have identified some odor-active volatiles in 'Marion' and 'Thornless Evergreen' blackberries (Klesk and Qian, 2003a, 2003b), but most analyses of blackberry volatiles investigated fresh or processed 'Evergreen' cultivars (Scanlan et al., 1970; Houchen et al., 1972; Gulan et al., 1973; Georgilopoulos and Gallois, 1987a, 1987b, 1988; Humpf and Schreier, 1991; Herrmann, 1992; Li et al., 1998). Accordingly, the purpose of this investigation was to identify and compare the volatile compositions of the two cultivars using capillary gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS).

MATERIALS & METHODS

Chemicals

Authentic aroma standards were obtained as follows: butyl acetate, limonene, octyl acetate, 2-heptanone, and 2-undecanone (K&K Laboratories, Jamaica, NY). Acetic acid, β-ionone, butanoic acid, *l*-carvone, ethyl acetate, ethyl butanonate, ethyl hexanoate, ethyl 2-methylbutanoate, eugenol, 2-heptanol, hexanal, hexanoic acid, *t*-2-hexenal, linalool, 3-methylbutanal, 2-methylbutanoic acid, octanol, phenethyl alcohol (Aldrich Chemical Co. Inc., Milwaukee, WI).

Blackberry Samples

'Marion' and 'Thornless Evergreen' blackberries were grown in Woodburn, Oregon from 5-10 year-old plants. The fruits (both machine and hand-harvested), were washed, graded, individually quick-frozen (IQF), and stored at –18 °C. Fruit samples were obtained from year 1999, 2001, and 2002 growing seasons (year 2000 fruit was not available). Sample boxes of fruit (13.6 kg) were transported on ice to the laboratory, where they were stored at –23°C.

Extraction of Volatile Compounds

For each cultivar and each growing season, 500 grams of frozen blackberries were placed in a 1 L Erlenmeyer flask with 400 mL of freshly distilled pentane:diethyl ether (1:1 v/v). The berries were stirred for 3 hours at 150 rpm on a platform shaker (Innova 2300, New Brunswick Scientific, Edison, NJ), and the solvent and juice poured into a separatory funnel. The juice was drawn off and returned to the fruit, and the organic phase retained. The berries and juice were then stirred and extracted twice more using 150 mL portions of pentane:diethyl ether. The organic extracts were combined to yield a total volume of 600 mL. Non-volatiles were removed from the organic extract using solvent assisted flavor extraction (SAFE) at 50°C, under vacuum according to the method proposed by Engel and others (Engel et al., 1999). The SAFE extract was dried with anhydrous Na_2SO_4, concentrated to 2 mL by solvent evaporation, and reduced to its final volume of 0.2 mL with a flow of nitrogen. This extraction was done in triplicate, for each cultivar and growing season pairing (18 extractions total).

GC Analysis

The analysis was performed using a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector (FID). Samples were analyzed on a DB-Wax column (60 m x 0.32 mm i.d. cross-linked polyethylene glycol, 0.5 μm film thickness, J&W Scientific, Folsom, CA). Injector and detector temperatures were 250 °C, the helium column flow rate was 2.0 mL/min at 25 °C, and the 2 μL sample injections were splitless. The oven temperature was programmed for a 2 min hold at 40 °C, then 40 to 235 °C at 2 °C/min (30 min hold). Retention indices were estimated in accordance with a modified Kovats method (Van den Dool and Kratz, 1963).

GC-MS Analysis

To identify compound peaks obtained in the GC-FID analyses, the same samples (2 μL splitless injections) were analyzed using an Agilent 6890 gas chromatograph equipped with an Agilent 5973 Mass Selective Detector (MSD). System software control and data management/analysis was performed through Enhanced ChemStation Software, G1701CA v. C.00.01.08 (Agilent Technologies, Inc., Wilmington, DE). Volatile separation was achieved with the same DB-Wax capillary column used in the GC-FID analyses. The helium column flow rate was set to 2.0 mL/min at 25 °C, and constant pressure mode (15.72 psi). The oven temperature was programmed as for the GC-FID analysis. Injector, detector transfer line, and ion source temperatures were 250, 280, and 230 °C, respectively. Electron impact mass spectrometric data from m/z 35-300 was collected at 5.27 scans/s, at an ionization voltage of 70 eV. Retention indices were estimated in accordance with a modified Kovats method (Van den Dool and Kratz, 1963). Compound identifications were made by comparing Kovats retention indices (RI) to those

of authentic standards, RI reported in literature (J. Agric. Food Chem.; Rychlik et al., 1998; among others), and mass spectral data from the Wiley 275.L (G1035) Database (Agilent Technologies, Inc., Wilmington, DE).

RESULTS & DISCUSSION

Tables 4.1 and 4.2 list 'Marion' and 'Thornless Evergreen' blackberry volatiles separated with the 60 m DB-Wax column. The FID peak area percentages for each compound were averaged over the three growing seasons. Table 4.1 lists 78% of the recorded total area percentage of 'Marion', while Table 4.2 lists 90% of 'Thornless Evergreen'. Combined table data shows 106 volatiles were identified; 46 of these have not been previously reported in blackberry (Nijssen et al., 1996). Forty-seven of the 106 volatiles were common to both cultivars, and 13 of these shared volatiles were not previously reported in blackberry (Nijssen et al., 1996). Based on total percentage of FID area the 'Thornless Evergreen' contains significantly more alcohols, hydrocarbons, and phenols than 'Marion', while 'Marion' contains more acids and esters. In both cultivars alcohols were the most abundant volatiles.

Table 4.1: 'Marion' volatiles (DB-Wax) (GC-FID)

	Compound[a]	Growing season[b]				
RI	Acids (29.10)	2002	2001	1999	Area %[c]	std dev
1468	Acetic acid	17.37	6.27	8.72	10.79	5.83
1668	Butanoic acid *	2.67	1.12	0.63	1.47	1.06
1722	2-Methylbutanoic acid *	1.58	1.72	2.86	2.05	0.70
1875	Hexanoic acid *	12.12	8.77	10.86	10.58	1.69
1998	*t*-2-Hexenoic acid *	0.24	0.91	0.27	0.47	0.38
2094	Octanoic acid *	1.42	0.92	1.82	1.39	0.45
2311	Decanoic acid *	2.21	2.15	2.66	2.34	0.28
	Alcohols (32.34)					
955	Ethanol	15.95	15.03	20.38	17.12	2.86
1226	2-Methylbutanol	0.24	0.31	0.30	0.28	0.04
1228	3-Methylbutanol	0.22	0.21		0.22	0.00
1342	2-Heptanol	2.47	2.68	2.35	2.50	0.17
1377	Hexanol	1.21	2.71	1.34	1.75	0.83
1409	*cis*-3-Hexenol	1.06	0.80	0.92	0.93	0.13
1431	*t*-2-Hexenol	0.38	1.39	0.43	0.73	0.57
1489	*dl*-6-Methyl-5-hepten-2-ol *	0.21	0.31	0.22	0.25	0.06
1541	2-Nonanol *	0.42	0.35	0.37	0.38	0.04
1569	Linalool	1.25	3.45	4.12	2.94	1.50
1582	Octanol	1.11	0.63	0.72	0.82	0.25
1640	4-Terpineol	0.03	0.43	0.09	0.18	0.22
1707	Nonanol	0.17	0.21	0.24	0.20	0.03
1727	α-Terpineol	0.08	0.12	0.14	0.11	0.03
1742	2-Dodecanol *	0.53	0.57	0.60	0.57	0.04
1787	Decanol	0.33	0.30	0.32	0.32	0.02
1882	*p*-Cymen-8-ol	0.18	0.29	0.33	0.27	0.07
1913	Benzyl alcohol	0.90	1.22	1.18	1.10	0.18
1929	α-Ionol *	0.07	0.10	0.13	0.10	0.03
1950	Phenethyl alcohol	0.24	0.60		0.42	0.25
2030	4-Phenyl-2-butanol *	0.32	0.48	0.32	0.37	0.09
2335	Cinnamic alcohol	0.72	1.11	0.50	0.77	0.31

Table 4.1 (continued): 'Marion' volatiles (DB-Wax) ...

	Compound[a]	Growing season[b]				
RI	Aldehydes (1.48)	2002	2001	1999	Area %[c]	std dev
1099	Hexanal	0.99	0.96	0.29	0.75	0.39
1238	*t*-2-Hexenal	0.94	0.90	0.14	0.66	0.45
1552	Benzaldehyde	0.04	0.11	0.07	0.08	0.04
	Esters (8.86)					
904	Ethyl acetate	5.84	8.71	8.37	7.64	1.57
1248	Ethyl hexanoate *	0.23	0.12	0.05	0.13	0.09
1291	Hexyl acetate	0.30	0.22	0.12	0.21	0.09
1353	*t*-2-Hexenyl acetate	0.11	0.07	0.05	0.08	0.03
1453	Ethyl octanoate	0.08	0.06	0.05	0.06	0.01
1546	Ethyl 3-hydroxybutanoate *	0.02	0.26	0.02	0.10	0.14
1674	Ethyl decanoate	0.10	0.04	0.02	0.05	0.04
1811	Methyl salicylate *	0.73	0.23	0.16	0.37	0.31
1865	Ethyl dodecanoate	0.08	0.04	0.03	0.05	0.03
2283	Ethyl hexadecanoate	0.18	0.15	0.14	0.16	0.02
	Hydrocarbons (1.96)					
1033	α-Pinene	0.13	0.24	0.07	0.14	0.09
1058	Toluene *	0.19	0.12	0.14	0.15	0.04
1078	Camphene	0.06	0.23	0.04	0.11	0.11
1215	Limonene	0.21	0.38	0.11	0.23	0.13
1263	γ-Terpinene	0.06	0.14	0.05	0.08	0.05
1277	Styrene *	0.03	0.25	0.21	0.16	0.12
1301	α-Terpinolene	0.56	0.57	0.21	0.45	0.20
1632	β-Caryophyllene *	0.44	0.15	0.27	0.29	0.15
1769	α-Farnesene *	0.51	0.26	0.25	0.34	0.15
	Ketones (2.76)					
915	2-Butanone *	0.51	0.97	1.10	0.86	0.31
1201	2-Heptanone	0.69	0.25	0.19	0.38	0.27
1308	Acetoin	0.40	0.17	0.12	0.23	0.15
1416	2-Nonanone *	0.11	0.05	0.07	0.08	0.03
1626	2-Undecanone	0.96	0.70	1.16	0.94	0.23
1758	Verbenone	0.06	0.07	0.04	0.06	0.01
1894	α-Ionone	0.04	0.11	0.03	0.06	0.05
1977	β-Ionone	0.15	0.11	0.22	0.16	0.05

Table 4.1 (continued): 'Marion' volatiles (DB-Wax) ...

	Compound[a]	Growing season[b]				
RI	Miscellaneous (0.06)	2002	2001	1999	Area %[c]	std dev
1576	Theaspirane B *	0.04	0.09		0.06	0.03
	Phenols (0.19)					
2211	Eugenol	0.14	0.23	0.20	0.19	0.05
	Unknown (1.66)					
1115	Unk	0.20	0.25	0.25	0.23	0.03
1828	Unk	0.24	0.16	0.13	0.18	0.05
2075	Unk	0.28	0.49	0.33	0.37	0.11
2136	Unk	0.24	0.18	0.31	0.24	0.06
2215	Unk	0.29	0.28	0.36	0.31	0.04
2244	Unk	0.23	0.63	0.15	0.34	0.25

a (values) compound class total peak area %; b peak area average of 3 replicates

c average of 3 growing seasons

* = not previously reported in blackberry

Table 4.2: 'Thornless Evergreen' volatiles (DB-Wax) (GC-FID)

	Compound[a]	Growing season[b]				
RI	Acids (7.74)	2002	2001	1999	Area %[c]	std dev
1470	Acetic acid	3.53	0.89	3.42	2.62	1.49
1656	Butanoic acid *	0.18	0.06	0.08	0.10	0.06
1697	Isovaleric acid *	3.02	1.38	1.43	1.94	0.93
1876	Hexanoic acid *	2.63	2.70	1.94	2.42	0.42
2000	*t*-2-Hexenoic acid *	0.46	0.56	0.40	0.48	0.08
2090	Octanoic acid *	0.30	0.13	0.10	0.18	0.11
	Alcohols (57.99)					
954	Ethanol	15.27	1.22	4.02	6.84	7.44
1044	2-Butanol *	0.08	0.04	0.07	0.06	0.02
1055	2-Methyl-3-buten-2-ol	0.02	0.05	0.07	0.05	0.03
1112	Isobutyl alcohol *	1.09	0.22	0.24	0.52	0.50
1140	2-Pentanol *	0.14	0.10	0.12	0.12	0.02
1165	Butanol *	1.38	0.29	0.39	0.69	0.60
1180	1-Penten-3-ol *	0.37	0.47	0.33	0.39	0.07
1219	2-Methyl/3-Methylbutanol *	0.08	0.04	0.04	0.05	0.02
1272	3-Methyl-3-buten-1-ol *	0.44	0.16	0.40	0.33	0.15
1348	2-Heptanol	9.75	18.96	21.86	16.85	6.32
1379	Hexanol	9.55	5.13	2.89	5.86	3.39
1389	*t*-3-Hexenol *	0.19	0.09	0.06	0.11	0.07
1410	*cis*-3-Hexenol	0.51	0.52	0.58	0.54	0.04
1432	*t*-2-Hexenol	1.08	1.54	1.16	1.26	0.25
1441	*cis*-2-Hexenol *	0.05	0.06	0.04	0.05	0.01
1481	Heptanol	0.31	0.24	0.26	0.27	0.04
1487	*dl*-6-Methyl-5-hepten-2-ol *	0.04	0.06	0.08	0.06	0.02
1539	2-Nonanol *	0.03	0.01	0.15	0.06	0.08
1565	Linalool	0.06	0.04	0.06	0.05	0.01
1570	Octanol	0.71	0.46	0.62	0.60	0.13
1586	2,3 Butanediol *	10.32	4.66	2.86	5.95	3.89
1634	4-Terpineol	0.29	0.83	1.08	0.73	0.40
1659	1-Terpineol *	0.06	0.19	0.18	0.14	0.08
1687	Nonanol	0.54	0.43	0.36	0.45	0.09
1718	1,8 Menthadien-4-ol *	0.03	0.05	0.06	0.04	0.01
1731	α-Terpineol	1.84	4.10	3.68	3.20	1.20
1743	*l*-Borneol	0.19	0.01		0.10	0.13
1790	Decanol	0.91	1.15	0.76	0.94	0.20

Table 4.2 (continued): 'Thornless Evergreen' volatiles ...

	Compound[a]	Growing season[b]				
RI	Alcohols cont. (57.99)	2002	2001	1999	Area %[c]	std dev
1794	Citronellol *	0.06	0.09	0.08	0.08	0.02
1829	Nopol *	1.66	4.74	3.81	3.41	1.58
1833	Myrtenol	0.19	0.18	0.15	0.17	0.02
1883	*p*-Cymen-8-ol	1.45	4.38	4.42	3.42	1.70
1914	Benzyl alcohol	0.69	0.92	0.80	0.81	0.12
1951	Phenethyl alcohol	0.99	2.14	1.81	1.65	0.59
2031	4-Phenyl-2-butanol *	0.07	0.10	0.06	0.08	0.02
2047	Perilla alcohol	0.70	1.03	0.92	0.89	0.17
2146	*p*-Cymen-α-ol *	0.20	0.79	0.65	0.54	0.31
2336	Cinnamic alcohol	0.53	0.80	0.56	0.63	0.14
	Aldehydes (1.27)					
927	2-Methylbutanal *	0.04	0.04	0.03	0.04	0.01
930	3-Methylbutanal	0.07	0.04	0.04	0.05	0.02
1100	Hexanal	0.17	0.38	0.14	0.23	0.13
1117	2-Methyl-2-butenal *	0.01	0.09	0.11	0.07	0.05
1239	*t*-2-Hexenal	0.60	1.07	0.26	0.64	0.41
1521	2,4 Heptadienal	0.03	0.06	0.09	0.06	0.03
1665	Myrtenal	0.09	0.24	0.20	0.18	0.08
	Esters (4.65)					
905	Ethyl acetate	3.96	1.66	4.85	3.49	1.64
1051	Ethyl butanoate	0.01			0.01	0.00
1067	Ethyl 2-methylbutanoate	0.05	0.01	0.02	0.03	0.02
1083	Butyl acetate	0.06	0.01	0.01	0.03	0.03
1252	Ethyl hexanoate *	0.40	0.07	0.06	0.18	0.20
1281	Hexyl acetate	0.01	0.02	0.03	0.02	0.01
1355	*t*-2-Hexenyl acetate	0.03	0.04	0.03	0.03	0.01
1367	Ethyl hexenoate	0.05	0.01	0.01	0.02	0.02
1452	Ethyl octanoate	0.01	0.03	0.02	0.02	0.01
1491	Octyl acetate	0.08	0.18	0.14	0.13	0.05
1547	Ethyl 3-hydroxybutanoate *	0.36	0.02	0.04	0.14	0.19
1661	Ethyl decanoate	0.06			0.06	0.00
1814	Methyl salicylate *	0.23	0.35	0.53	0.37	0.15
1869	Ethyl dodecanoate	0.04	0.05	0.04	0.04	0.01
2284	Ethyl hexadecanoate	0.11	0.08	0.04	0.08	0.03

Table 4.2 (continued): 'Thornless Evergreen' volatiles ...

	Compound[a]	Growing season[b]				
RI	Hydrocarbons (8.78)	2002	2001	1999	Area %[c]	std dev
1035	α-Pinene	1.23	5.71	5.67	4.20	2.58
1059	Toluene *	0.73	0.47	0.51	0.57	0.14
1079	Camphene	0.14	0.40	0.49	0.34	0.18
1127	β-Pinene	0.03	0.03	0.03	0.03	0.00
1191	α-Phellandrene	0.19	0.22	0.24	0.22	0.03
1216	Limonene	0.31	0.98	0.92	0.74	0.37
1230	Sabinene *	2.76	0.50	0.48	1.25	1.31
1264	γ-Terpinene	0.04	0.09	0.06	0.06	0.03
1277	Styrene *	0.01	0.16	0.23	0.13	0.11
1290	*p*-Cymene *	0.21	0.70	0.66	0.52	0.27
1303	α-Terpinolene	0.30	0.87	0.51	0.56	0.29
1461	1-Methyl-4-isopropenylbenzene*	0.07	0.23	0.19	0.16	0.08
	Ketones (2.37)					
916	2-Butanone *	0.23	0.06	0.16	0.15	0.09
996	2-Pentanone	0.09	0.04	0.04	0.06	0.03
1010	3-Methyl-3-buten-2-one *	0.14	0.20	0.23	0.19	0.05
1202	2-Heptanone	2.84	1.48	0.84	1.72	1.02
1309	Acetoin	0.29	0.04	0.07	0.13	0.14
1553	Camphor	0.03	0.05	0.08	0.05	0.02
1779	Carvone	0.02	0.10	0.09	0.07	0.04
	Miscellaneous (0.14)					
1496	Linalool oxide	0.09	0.04	0.02	0.05	0.04
1750	γ-Hexalactone	0.03	0.10	0.12	0.08	0.05

Table 4.2 (continued): 'Thornless Evergreen' volatiles ...

	Compound[a]	Growing season[b]				
RI	Phenols (1.93)	2002	2001	1999	Area %[c]	std dev
1901	Guaiacol *	0.01	0.07	0.05	0.04	0.03
2043	Phenol *	0.03	0.06	0.03	0.04	0.02
2048	Methyl eugenol	0.70			0.70	0.00
2212	Eugenol	0.55	0.69	0.67	0.64	0.08
2268	Elemicin	0.49	0.62	0.45	0.52	0.09
	Unkowns (6.00)					
1122	Unk		0.31	0.27	0.29	0.03
1228	Unk		0.84	0.64	0.74	0.14
1484	Unk	0.43	0.53	1.19	0.72	0.42
1593	Unk	0.14	0.20	0.15	0.16	0.03
1696	Unk		0.72	0.89	0.80	0.12
1738	Unk	0.56	1.02	1.48	1.02	0.46
1772	Unk	0.17	0.62	0.59	0.46	0.25
1947	Unk	0.38	1.18	0.68	0.75	0.40
2095	Unk	0.29	0.73	0.57	0.53	0.22
2315	Unk	0.11	0.38	0.16	0.22	0.14
2393	Unk	0.17	0.48	0.30	0.32	0.15

a (values) compound class total peak area %; b peak area average of 3 replicates

c average of 3 growing seasons

* = not previously reported in blackberry

In 'Marion' acids were the second most abundant volatile class, followed by esters, ketones, hydrocarbons, aldehydes, and phenols. In 'Thornless Evergreen' the second most abundant volatile class was hydrocarbons, followed by acids, esters, ketones, phenols, and aldehydes. The six most abundant volatiles in 'Marion' were ethanol, acetic acid, hexanoic acid, ethyl acetate, linalool, and 2-heptanol; they totaled 52% of total peak area. In 'Thornless Evergreen' the six most abundant volatiles were 2-heptanol, ethanol, 2,3 butanediol, hexanol, α-pinene, and ethyl acetate; they totaled 43% of total peak area.

The most abundant alcohols in 'Marion' were ethanol, linalool, 2-heptanol, and hexanol, while in 'Thornless Evergreen' 2-heptanol, ethanol, 2,3 butanediol, hexanol, *p*-cymen-8-ol, nopol, and α-terpineol were dominant. The dominant acids in 'Marion' were acetic, hexanoic, decanoic, and 2-methylbutanoic; in 'Thornless Evergreen' they were acetic, hexanoic, and isovaleric. Primary hydrocarbons in 'Marion' were α-terpinolene, α-farnesene, β-caryophyllene, and limonene; in 'Thornless Evergreen' they were α-pinene, sabinene, limonene, and α-terpinolene. Hexanal and *t*-2-hexenal were the dominant aldehydes in 'Marion'; 'Thornless Evergreen' contained *t*-2-hexenal, hexanal, and myrtenal. Ethyl acetate and methyl salicylate were the primary esters in both cultivars. Dominant 'Marion' ketones and phenols were 2-undecanone, 2-butanone, and eugenol, while in 'Thornless Evergreen' they were 2-heptanone, methyl eugenol, eugenol, and elemicin.

The biogenesis of fruit volatiles is of interest to flavor chemists, plant breeders, and biotechnologists alike, as identifying the phytochemical origins of flavor compounds and their precursors provides insights on the composition and proportions of those fruit volatiles that define fruit smell. This phytochemical knowledge is used in parallel by these disciplines to identify,

develop, and enhance consumer-preferred olfactory qualities of fruit. Fruit flavor research has examined free volatiles and bound glycosidic precursors (i.e., bound volatiles) in grapes, fruit juices, and wine (Rouseff and Leahy, 1995). Research studies have examined volatile biogenesis in apples, kiwi, pineapple, strawberry, and tomato, and exotic fruits such as quince, passion fruit, and guava, et al. (Williams, 1993; Rouseff and Leahy, 1995). However, blackberry volatile metabolism has not been specifically addressed. Volatile biogeneration in plants uses three main chemical compound classes: fatty acids, amino acids, and carbohydrates.

In 'Marion' the major organic acids were even acids C_2 through C_{10}, in 'Thornless Evergreen' they were C_2, C_4, and C_6; they are derived from fatty acids. Fatty acids are thought to be the primary precursors of most plant volatiles, and in general are broken down by two oxidative pathways: β-oxidation and lipoxygenase (LOX) (Sanz et al., 1997). Beta-oxidation is thought to produce "primary aromas", those generated in intact fruits. Enzymes in the β-oxidation cycle metabolize fatty acid acyl-CoA derivatives to shorter chain acyl-CoAs. The oxidation cycle involves, in order, acyl-CoA dehydrogenase (with FAD), enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase (with NAD), and acetyl-CoA acetyltransferase (thiolase, with free CoA). This enzymatic series generates acetyl-CoA and an acyl-CoA shorter by two carbons. The various resulting acyl-CoAs are converted into esters via alcohol acyltransferase (Paillard, 1979).

(E)-2-hexenoic acid is the major unsaturated fatty acid found in both cultivars. Unsaturated fatty acids require auxiliary enzymes to complete oxidation. In the case of a C_n unsaturated fatty acid-CoA, β-oxidation yields a C_{n-x1} (Z)-3-enoyl-CoA ($x1$ = number of carbons removed by oxidation down to the first double bond), which is isomerized by enoyl-CoA isomerase to C_{n-x1}

(E)-2-enoyl-CoA . (E)-2-enoyl-CoA is the natural substrate for enoyl-CoA hydratase (Sanz et al., 1997). In the case of polyunsaturated fatty acids, the oxidation is similar to that for an unsaturated fatty acid, but produces a C_{n-x2} (Z)-2-enoyl-CoA ($x2$ = number of carbons removed by oxidation down to the last double bond). This molecule is hydrated by enoyl-CoA hydratase to R-(-)-3-hydroxyacyl-CoA, not the (S)-(+)-enantiomer produced from saturated fatty acids. Conversion to the (S)-(+) isomer, which is the natural substrate of 3-hydroxyacyl-CoA dehydrogenase, is made via 3-hydroxyacyl-CoA epimerase (Sanz et al., 1997). It is thought these two auxiliary enzymes may account for the different enantiomeric ester compositions in tropical fruits (Tressl et al., 1985).

The lipoxygenase (LOX) pathway is thought to produce "secondary aromas" from the disruption of plant tissues, either from crushing, slicing, and the like, or from fruit ripening (Sanz et al., 1997). In both cultivars this pathway is the source of the "green" aroma of *cis*-3-hexenol and the green-fruity aroma of *t*-2-hexenol, et al. The LOX pathway is preceded by the action of acylhydrolases, which free polyunsaturated fatty acids from glycolipids, phospholipids, or triacylglycerols. LOX degradation of linoleic and linolenic acids generates many fruit acids, alcohols, aldehydes, and esters (Stone, et al., 1975; Olias et al., 1993; Perez et al., 1999). LOX degradation of linoleic and linolenic acids proceeds first via LOX isozymes to produce fatty acid hydroperoxides, preferentially at C_9 or C_{13}, or non-specifically at either carbon. In turn these hydroperoxides are converted to aldehydes and oxoacids via hydroperoxide lyase (HL) (Sanz et al., 1997).

Three classes of HL, C_9, C_{13}, or non-specific, determine aroma composition in many plants, despite the specific action of LOX present. For example, pear LOX forms mainly 13-hydroperoxides, but pear HL is the C_9 class. Olive LOX is the C_9 class, but its HL is the C_{13} class. This specificity of

olive HL is evidenced by the almost total absence of C_9 carbonyls, and high content of C_6 alcohols, aldehydes, and esters in virgin olive oil aroma volatiles (Olias et al., 1993). Similarly, cucumber LOX produces C_9 : C_{13} : non-specific hydroperoxides in the ratio of 13 : 9/85 : 15, and its HL is non-specific, which accounts for the presence of C_9 carbonyls important to cucumber aroma (Galliard et al., 1976; Wardale and Lambert, 1980).

Hydroperoxide lyase activity on 13-hydroperoxylinoleic acid or 13-hydroperoxylinolenic acid produces 12-oxo-(9Z)-dodecenoic acid and hexanal or (3Z)-hexenal respectively. HL activity on the corresponding 9-hydroperoxides of these fatty acids yields 9-oxononanoic acid and (3Z)-nonenal or (3Z, 6Z)-nonadienal respectively (Sanz et al., 1997). Most plants isomerize compounds with a (3Z)-enal structure to the (2E)-enal form with a (3Z, 2E)-enal isomerase (Sanz et al., 1997). In most plants, the unsaturated aldehydes produced by HL are reduced by alcohol dehydrogenase to their corresponding alcohols, either before or after isomerization. These alcohols are natural substrates for alcohol acyltransferase, to produce esters (Sanz et al., 1997).

In fruits, amino acids are direct precursors of volatile compounds, and when metabolized generate aliphatic, aromatic, or branched acids, alcohols, carbonyls, and esters (e.g., 2/3-methylbutanoic acid in both cultivars; Sanz et al., 1997). It has been shown that variations in free amino acid content occur during fruit ripening, when characteristic aroma is produced. This implies that different fruit aroma profiles could be related to a free amino acid pool (Tressl and Drawert, 1973). Amino acids are transformed using three enzymatic classes: aminotransferase, decarboxylase, and alcohol dehydrogenase. The first step uses an amino acid-specific 2-oxoglutarate aminotransferase to produce a 2-oxoacid from the amino acid. The decarboxylation of the 2-

oxoacid is thought to occur via either an enzymatic complex similar to that of pyruvate dehydrogenase (decarboxylating), or 2-oxoglutarate dehydrogenase from the Krebs cycle (Sanz et al., 1997). The various aldehydes produced may then be transformed into alcohols or acids with alcohol dehydrogenase or aldehyde oxidase, respectively. Acyl-CoA products from the action of 2-oxoglutarate dehydrogenase are transformed into esters via alcohol acyltransferase. Evidence exists to indicate decarboxylation final products may depend on the plant species (Sanz et al., 1997).

A different volatile metabolic pathway has been proposed using aromatic amino acid (tyrosine and phenylalanine) precursors, leading to compounds with phenolic and spicy odors. Cinnamic acid, derived from phenylalanine via phenylalanine ammonia lyase (PAL), and *p*-coumaric acid, derived from tyrosine via PAL, or from cinnamic acid via cinnamic acid hydrolase, are suggested as starting intermediates for this pathway (Tressl and Albrecht, 1986; Sanz et al., 1997). Cinnamic acid, through loss of acetate, leads to benzoic acid and its derivatives, while *p*-coumaric acid, converted to caffeic acid by phenolase, leads to phenolic derivatives (Sanz et al., 1997). Cinnamic alcohol, present in both cultivars, may be generated from the reduction of cinnamic acid.

While fruit aromas are predominantly based on ester composition, fruits may also use amino acid substrates similarly as vegetables to produce sulfur-containing volatiles with aromas that are vegetal rather than fruity. Free amino acids are indirect precursors of vegetal aromas, as they are metabolized into derivative compounds that in turn are enzymatically converted to aroma compounds with cell disruption (Chin and Lindsay, 1994). Two of the major classes of these compounds are the S-alk(en)yl-cysteine sulfoxides and glucosinolates. The S-alk(en)yl-cysteine sulfoxides are precursors to the

characteristic aroma of *Allium* and *Brassica* species. A proposed pathway suggests cysteine and serine as precursors for the sulfoxides, and the key enzymatic step in aroma generation is accomplished by alliinase (alliin alkyl-sulfenate lyase). Upon cell disruption S-alk(en)yl-cysteine sulfoxides in the cytoplasm are spilt by alliinases released from vacuoles to produce dialk(en)yl thiosulfinates. These thiosulfinates are unstable and undergo rapid spontaneous non-enzymatic reactions to form numerous volatile sulfurous compounds characteristic of *Allium* and *Brassica* species (Sanz et al., 1997). Although GC-FID analysis of 'Marion' and 'Thornless Evergreen' did not identify any sulfurous compounds, other studies identified six sulfur volatiles with alliaceous and vegetal aromas in both cultivars (Klesk and Qian, 2003a, 2003b.)

Glucosinolates are sulfur compounds whose breakdown products contribute to the flavor of the *Cruciferae* family of plants, but little is known about their biosynthesis. Plants that contain glucosinolates also contain enzymes that degrade them. These enzymes, thioglucoside glucohydrolases, catalyze the hydrolysis of the thioglucosidic linkage in glucosinolates. The released aglucones undergo non-enzymatic reactions to produce volatiles. It is assumed that enzymes and substrates are segregated from one another until cell disruption, as for the S-alk(en)yl-cysteine sulfoxides (Sanz et al., 1997).

Relatively few aroma compounds derive from carbohydrates. Fruit terpenes (mainly monoterpenes) are produced from carbohydrates through the isoprenoid pathway (Sanz et al., 1997). Both cultivars contain relatively small amounts (by relative peak area) of camphene, limonene, α-pinene, and α-terpinolene. Mevalonic acid is considered the first specific terpene precursor, and is used to produce isopentyl diphosphate (IPP), the hypothetical 'active isoprene' unit from which all isoprenoid compounds derive. IPP is produced by the sequential double phosphorylation of mevalonic acid by mevalonate

kinase and 5-phosphomevalonate kinase to produce mevalonic acid diphosphate (MVAPP). IPP is then produced by the decarboxylation and dehydration of MVAPP by MVAPP decarboxylase (Sanz et al., 1997). In order to produce geranyl diphosphate (GPP), the direct precursor of monoterpenes, one molecule of IPP is isomerized to the dimethylallyldiphosphate form (DMAPP) by isopentenyl diphosphate isomerase. Prenyltransferases then produce GPP by the condensation of DMAPP and IPP. Monoterpenes are then produced from GPP through hydrolysis, cyclations (key step), and oxidoreductions (Sanz et al., 1997).

Furanones, five of which were identified in both cultivars, in another study, are another carbohydrate derived compound class important to fruit aromas. These compounds are the result of the Maillard reaction, the browning reaction of reducing sugars with amine salts (Klesk and Qian, 2003b; Schwab, 1998; Sanz et al., 1997). Despite the importance of furanones in fruit aroma, their biosynthesis is unclear. Studies were attempted to detail the formation pathway of 2,5-dimethyl-4-hydroxy-3-(2H)-furanone (furaneol), identified as an important aroma in many fruits including pineapple, mango, grapefruit, tomato, strawberry, raspberry, and blackberry (Sanz et al., 1997; Klesk and Qian, 2003b). A study demonstrated furaneol and its derivatives mesifurane (2,5-dimethyl-4-methoxy-3-(2H)-furanone) and furaneol acetate (2,5-dimethyl-4-acetoxy-3-(2H)-furanone) were formed by direct conversion of D-fructose in a biological Maillard reaction (Schwab, 1998). Stable isotope ratio analysis suggests a pathway that converts D-fructose to 1-deoxyfructose or 6-deoxyfructose, which are in turn converted to furaneol, probably through dehydration and reduction reactions. This is contrary to an earlier proposal that furaneol is formed by the coupling of two C_3 units (Schwab, 1998).

Some final comments address ester formation in fruits. Esters constitute the main group of compounds identified in fruit aroma, and are produced by the esterification of alcohols and carboxylic acids. Biogeneration of these precursor alcohols and acids are generally well explained by the enzymatic actions on lipids and amino acids previously discussed. However, little is known about the actual esterification reaction itself. In micro-organisms two enzymes are implicated in ester formation: alcohol acyltransferase (AAT) and esterase. AAT catalyzes the transfer of an acyl moiety of an acyl-CoA onto the corresponding alcohol, while esterase hydrolyzes esters. These enzymatic activities have been described in fruits (Sanz et al., 1997). Research information indicates that major factors in ester biogeneration include fruit ripening, availability of substrates, and the substrate specificity of alcohol acyltransferases for both the acyl moieties of acyl-CoAs, and the corresponding alcohols (Sanz et al., 1997). Ten of fifteen esters identified in this study were ethyl esters. Seven of ten esters shared by both cultivars were ethyl esters; even esters C_2 through C_{12}, and C_{16}.

Since the aroma profile of fruit is a function of volatile concentration, odor thresholds, and odor activity values, among others, only conjecture may be made concerning the potential contributions to blackberry flavor of each volatile compound class listed in the Tables. Assuming each compound class "odor activity" is the same for each cultivar, the fact that 'Thornless Evergreen' contains approximately 1.75 times more alcohols (with green, camphoraceous, and minty descriptors), 4.5 times more hydrocarbons (herbaceous, camphoraceous, terpeny), and 9.5 times more phenolics (warm spices, clove, cinnamon) appears to support 'Thornless Evergreen' aroma already described. Similarly, 'Marion' has twice the esters (intensely fruity, floral), which supports its described aroma, but 4 times the organic acids (pungent, cheesy, citrus) of 'Thornless Evergreen', which contradicts the flavor conjecture. Recent gas

chromatography-olfactometry (GC-O, OSME) and aroma extract dilution analysis (AEDA) of 'Marion' and 'Thornless Evergreen' blackberries (Klesk and Qian, 2003a, 2003b) suggest the following volatiles may significantly contribute to 'Marion' aroma: 2-heptanol, linalool, and α-terpineol (collectively sweet, floral, and minty), hexanal (green, grassy), ethyl hexanoate (intensely fruity), α-pinene (piney), 2-heptanone and 2-nonanone (fruity, floral), and the theaspiranes (woody, camphoraceous). Similarly, 'Thornless Evergreen' aroma may include 3-methylbutanol, 2-heptanol, linalool, octanol, and α-terpineol (pungent, floral, citrus, minty), hexanal and t-2-hexenal (green, grassy), ethyl 2-methylbutanoate and ethyl hexanoate (green-fruity), α-pinene (piney), and 2-heptanone and *l*-carvone (fruity-spicy, minty). These facts strengthen the hypothesis that blackberry aroma is the result of a complex formulation of odor-active volatiles. This formulation may be significantly different within cultivars, while its aroma impact may also be affected by demonstrated sensory interactions affecting flavor perception, for example, Brix-acid ratios. Further study is required to clarify and quantify which subset of identified volatiles defines blackberry aroma, and their differences within cultivars.

CONCLUSION

Volatile compositional analysis of 'Marion' and 'Thornless Evergreen' generates data that provides insights on their phytochemical origins. These insights in turn suggest metabolic pathways for each cultivar's aroma compounds and precursors, which then may be used to develop and enhance preferred olfactory qualities of blackberry. The aroma profiles of 'Marion' and 'Thornless Evergreen' blackberries are complex, and continued study (gas chromatography-olfactometry, odor activity values (OAVs), aroma compound quantification) is required to clarify their specific compositions and further characterize cultivar differences.

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CHAPTER 5. CONCLUSION

'Marion' and 'Thornless Evergreen' blackberry aromas were compared using a pair of extraction and GC-O-MS methods. One method is based on purge-and-trap (P&T, dynamic headspace) extraction and aroma intensity rating by detection frequency (DetF) and a standard scale, and the other based on solvent-distillation (SAFE) extraction and aroma threshold (AEDA) measures. Table 5.1 summarizes the volatiles detected in the two blackberry cultivars, and clearly shows that these relative measures of volatile odor intensity cannot be positively correlated. This lack of correlation stems from the basis for the different measuring scales used, the different sample preparations, and the well documented variability in assessor GC-O performance. Eighty-four compounds were identified, twenty-one of them tentatively, and thirty-seven have not been previously reported in blackberry. Of the thirty-seven new volatiles, eight were unique to 'Marion', and one unique to 'Thornless Evergreen'. Further, fourteen of the thirty-seven have been previously reported in red raspberry; of these, five were unique to 'Marion', and nine were in both cultivars. "Marion' contained seventy-seven of eighty-four volatiles, and 'Thornless Evergreen' sixty-eight.

Table 5.1: Composite of 'Marion' and 'Thornless Evergreen' aroma

Compound[a]	GC-O[b] Method	Polar intensity[c] Marion	Polar intensity[c] Evergreen	Non-polar intensity[c] Marion	Non-polar intensity[c] Evergreen
Acetaldehyde*[r]	AEDA	-	-	1	8
	DetF	8	6	6	6
Benzaldehyde[r] [(T)]	AEDA	-	-	64	256
	DetF	-	-	10	10
2,3-Butanedione (Diacetyl)*[r]	AEDA	2	2	2	2
	DetF	12	11	11	10
l-Carvone	AEDA	8	4	2	-
	DetF	-	11	-	6
β-Damascenone[r] [(T)]	AEDA	4	32	8	4
	DetF	10	8	7	8
Dimethyltrisulfide*	AEDA	2	16	16	-
	DetF	11	-	8	-
Ethyl hexanoate[r]	AEDA	8	-	32	1
	DetF	7	6	8	8
Ethyl 2-methyl/3-methylbutanoate[r]	AEDA	4	-	128	1024
	DetF	10	10	-	-
Eugenol[r]	AEDA	-	-	4	4
	DetF	-	-	-	6
2-Heptanol[r]	AEDA	4	512	8	2
	DetF	11	9	12	-
2-Heptanone[r]	AEDA	16	32	-	-
	DetF	8	6	-	-
Hexanal[r]	AEDA	-	4	64	1024
	DetF	7	8	9	10
t-2-Hexenal[r]	AEDA	-	-	1	4
	DetF	-	7	10	10
Hexyl acetate[r]	AEDA	-	-	1	1
	DetF	-	-	8	7
Linalool[r]	AEDA	4	128	16	8
	DetF	11	8	11	9
Methional*	AEDA	32	512	256	2048
	DetF	8	7	-	11
3-Methylbutanal	AEDA	-	4	1	1
	DetF	8	-	-	10
Methyl hexanoate[r]	AEDA	8	4	-	-
	DetF	10	10	-	-

Table 5.1 (continued): Composite of 'Marion' and 'Thornless ...

Compound[a]	GC-O[b] Method	Polar intensity[c] Marion	Polar intensity[c] Evergreen	Non-polar intensity[c] Marion	Non-polar intensity[c] Evergreen
2-Methylpropanal* [(T)]	AEDA	-	-	1	-
	DetF	-	-	7	7
Nonanal[r]	AEDA	2	-	8	8
	DetF	10	7	10	9
t-2-Nonenal*	AEDA	-	-	1	64
	DetF	-	-	-	9
neo-allo-Ocimene* [(T)]	AEDA	-	-	16	4
	DetF	-	-	7	-
1-Octen-3-one*[r]	AEDA	8	16	2	16
	DetF	9	10	10	9
α-Terpinolene[r] [(T)]	AEDA	-	-	1	64
	DetF	-	-	7	7
Theaspirane A*[r] [(T)]	AEDA	4	-	2	1
	DetF	5	6	-	-
Theaspirane B*[r] [(T)]	AEDA	4	-	-	-
	DetF	8	7	-	-
2-Undecanone[r]	AEDA	8	128	4	16
	DetF	-	-	8	8
Benzyl acetate* [r]	AEDA	-	-	-	-
	DetF	10	-	-	-
Camphene [r]	AEDA	-	-	-	-
	DetF	-	-	-	7
cis-3-Hexenol [r]	AEDA	-	-	-	-
	DetF	-	5	-	-
Hexyl hexanoate*	AEDA	-	-	-	-
	DetF	-	-	8	6
Methyl 2-methylbutanoate*	AEDA	-	-	-	-
	DetF	8	-	-	-
3-Methylbutanol [r]	AEDA	-	-	-	-
	DetF	-	8	-	-
2-Methylpropanol* [r]	AEDA	-	-	-	-
	DetF	7	-	-	-
β-Myrcene* [r]	AEDA	-	-	-	-
	DetF	8	-	-	-
Myrtenol [r]	AEDA	-	-	-	-
	DetF	-	-	-	9

Table 5.1 (continued): Composite of 'Marion' and 'Thornless ...

Compound[a]	GC-O[b] Method	Polar intensity[c] Marion	Polar intensity[c] Evergreen	Non-polar intensity[c] Marion	Non-polar intensity[c] Evergreen
2-Nonanone* [r]	AEDA	-	-	-	-
	DetF	9	-	-	-
Nonyl acetate*	AEDA	-	-	-	-
	DetF	10	-	-	-
allo-Ocimene*	AEDA	-	-	-	-
	DetF	-	10	9	-
t-β-Ocimene*	AEDA	-	-	8	2
	DetF	-	7	-	-
Octanal	AEDA	-	-	-	-
	DetF	-	5	-	-
2-Octenal	AEDA	-	-	-	-
	DetF	-	-	6	7
Pentanal	AEDA	-	-	-	-
	DetF	-	-	7	6
1-Penten-3-one*	AEDA	-	-	-	-
	DetF	-	-	11	10
α-Phellandrene [r]	AEDA	-	-	-	-
	DetF	-	-	7	-
2-Phenylethylacetate*	AEDA	-	-	-	-
	DetF	-	-	6	-
α-Pinene [r]	AEDA	-	-	-	-
	DetF	8	9	-	6
β-Pinene [r]	AEDA	-	-	-	-
	DetF	-	-	9	11
γ-Terpinene [r]	AEDA	-	-	-	-
	DetF	-	-	5	-
α-Terpineol [r]	AEDA	-	-	-	-
	DetF	9	10	-	-
4-Terpineol [r]	AEDA	-	-	-	-
	DetF	-	-	-	6
Acetic acid[r]	AEDA	16	32	-	16
	DetF	-	-	-	-
Benzyl alcohol[r] [(T)]	AEDA	2	2	1	2
	DetF	-	-	-	-
Butanoic acid*[r]	AEDA	32	-	1	2
	DetF	-	-	-	-

Table 5.1 (continued): Composite of 'Marion' and 'Thornless ...

Compound[a]	GC-O[b] Method	Polar intensity[c] Marion	Polar intensity[c] Evergreen	Non-polar intensity[c] Marion	Non-polar intensity[c] Evergreen
Cinnamic alcohol[r] [T]	AEDA	8	32	-	-
	DetF	-	-	-	-
Cinnamic aldehyde [T]	AEDA	4	-	-	-
	DetF	-	-	-	-
Dimethyldisulfide*	AEDA	2	-	32	2048
	DetF	-	-	-	-
Dimethylsulfide* [T]	AEDA	16	128	16	8
	DetF	-	-	-	-
Ethyl acetate[r]	AEDA	16	2	1	-
	DetF	-	-	-	-
Ethyl 2-methylpropanoate*	AEDA	-	256	-	2048
	DetF	-	-	-	-
2,5-dimethyl-4-hydroxy-3(2*H*)-furanone*[r]	AEDA	8	1024	32	-
	DetF	-	-	-	-
4,5-dimethyl-3-hydroxy-2(5*H*)-furanone* [T]	AEDA	4	128	32	4
	DetF	-	-	-	-
2-ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone* [T]	AEDA	32	16	2	2
	DetF	-	-	-	-
5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone* [T]	AEDA	4	256	1	-
	DetF	-	-	-	-
4-hydroxy-5-methyl-3(2*H*)-furanone* [T]	AEDA	32	8	4	-
	DetF	-	-	-	-
Hexanoic acid*[r]	AEDA	2	128	-	-
	DetF	-	-	-	-
Hexanol[r] [T]	AEDA	4	-	-	-
	DetF	-	-	-	-
β-Ionone[r]	AEDA	-	-	2	16
	DetF	-	-	-	-
Limonene[r]	AEDA	-	-	4	2
	DetF	-	-	-	-
Methyl butanoate	AEDA	4	-	-	-
	DetF	-	-	-	-
2-Methylbutanoic acid*[r]	AEDA	32	128	32	16
	DetF	-	-	-	-
Methylethylsulfide* [T]	AEDA	-	-	16	8
	DetF	-	-	-	-

Table 5.1 (continued): Composite of 'Marion' and 'Thornless ...

Compound[a]	GC-O[b] Method	Polar intensity[c] Marion	Polar intensity[c] Evergreen	Non-polar intensity[c] Marion	Non-polar intensity[c] Evergreen
2-Methylthiophene* (T)	AEDA	-	-	32	512
	DetF	-	-	-	-
1-Octen-3-ol	AEDA	4	-	-	64
	DetF	-	-	-	-
Octyl acetate	AEDA	4	-	-	-
	DetF	-	-	-	-
Phenethyl alcohol[r]	AEDA	8	64	-	32
	DetF	-	-	-	-
Thiophene* (T)	AEDA	8	128	4	2048
	DetF	-	-	-	-
4-Vinylguaiacol*[r] (T)	AEDA	-	-	1	-
	DetF	-	-	-	-
Butyl acetate	AEDA	2	-	-	-
	DetF	9	-	-	-
Elemicin (T)	AEDA	-	-	1	-
	DetF	-	-	-	5
Ethyl butanoate	AEDA	4	-	2	-
	DetF	8	-	6	-
p-Methylacetophenone	AEDA	-	32	2	8
	DetF	-	7	-	-
Octanol[r]	AEDA	2	-	-	-
	DetF	-	10	-	-
Octyl formate*	AEDA	-	-	1	-
	DetF	-	-	-	7

a * = not previously reported in blackberry, r = reported in red raspberry, T = tentative identification

b AEDA = aroma extract dilution analysis, DetF = detection frequency

c polar (Stabilwax), non-polar (DB5); AEDA = 2^n, n = maximum dilution where odor still detected.

c DetF: 16 point scale, 0 = not detected, 15 = extreme intensity impact

Results show characteristic effects of extraction methods used. Twenty-seven of the eighty-four volatiles were not detected by the P&T-DetF method. Listed in Table 5.1 (pages 108-112), these volatiles were probably not detected for any of the following reasons. If the volatiles are very water soluble (e.g., furanones), or very water insoluble (e.g., sulfur compounds, hydrocarbons, C_7 and greater aromatics), P&T can discriminate against the extraction of these volatiles because of their correspondingly low vapor pressures. This discrimination is prominent when P&T results are compared to that of AEDA; all five furanones and five sulfur compounds not detected by P&T were detected by AEDA. Further, some small ($C_2 - C_6$) acids, alcohols, and esters, with different water solubilities, were not detected by P&T, but were detected in varying degrees by AEDA. These results reflect characteristic extraction differences. These small volatiles may not be detected in P&T due to breakthrough from the Tenax® trap, but may be detected in AEDA because of sample concentration effects. Finally, the non-detection of these volatiles may also be due to their concentrations not meeting the detection thresholds of the assessors. This explanation is compelling for those volatiles not detected by P&T, and only weakly detected by AEDA; that is, reported once or twice out of four opportunities (two columns x two cultivars).

Twenty-four of the eighty-four identified volatiles were not detected by AEDA. Listed in Table 5.1 (pages 108-112), these volatiles were probably not detected for the same reasons described above. For all twenty-four volatiles, there were only weak corresponding detections by P&T-DetF. Twelve of these twenty-four volatiles (three alcohols, four esters, three aldehydes, two ketones) were $C_4 - C_9$ compounds, and their weak detection may be due to solvent solubility and detection threshold issues. However, eleven hydrocarbons were not detected by AEDA, and nine of them were weakly detected by P&T-DetF. Since one expects relatively good to excellent extractions of hydrocarbons with

organic solvents, the data implies that these compounds' olfactory intensities in either method are just at the assessors' average detection thresholds for them. The qualitative implications of this to blackberry aroma is that these compounds are probably minor components, and provide only subtle background scents which refine and balance overall blackberry aroma.

Although the results obtained from the two extraction and analysis methods cannot be correlated, they can be overlapped and compared to generate a list of suspected significant odor-active blackberry volatiles. The most significant (FD≥16) odor-active volatiles in 'Marion' determined on the non-polar (DB-5) column were methional (FD = 256); ethyl 2-methylbutanoate (FD = 128); benzaldehyde and hexanal (FD = 64); 2-methylbutanoic acid, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, 4,5-dimethyl-3-hydroxy-2(5*H*)-furanone, ethyl hexanoate, dimethyldisulfide, and 2-methylthiophene (FD = 32); linalool, neo-allo-ocimene, dimethylsulfide, dimethyltrisulfide, and methylethylsulfide (FD = 16). Using the P&T-DetF intensity measure, diacetyl (DetF = 11), 2-heptanol (DetF = 12), *t*-2-hexenal (DetF = 10), hexyl acetate (DetF = 8), nonanal (DetF = 10), 1-octen-3-one (DetF = 10), 2-undecanone (DetF = 8), hexyl hexanoate (DetF = 8), allo-ocimene (DetF = 9), and β-pinene (DetF = 9) may also have significant odor impact, as their intensity ratings are moderately high or greater (DetF = 8). In addition, 2-ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone, 4-hydroxy-5-methyl-3(2*H*)-furanone, and butanoic acid (FD = 32), ethyl acetate, acetic acid, and 2-heptanone (FD = 16) may also be important to 'Marion' blackberry flavor, as they had high flavor dilution factors as determined on the polar (Stabilwax) column. Further, on the polar column β-damascenone (DetF = 10), methyl hexanoate (DetF = 10), benzyl acetate (DetF = 10), methyl 2-methylbutanoate (DetF = 8), 2-nonanone (DetF = 9), nonyl acetate (DetF = 10), α-pinene (DetF = 8), α-terpineol (DetF = 9), and butyl acetate (DetF = 9) may also contribute to 'Marion' flavor.

Many significant (FD≥16) odor-active volatiles in 'Thornless Evergreen' were identified on the DB-5 column. The most important aroma compounds included methional, ethyl 2-methylpropanoate, thiophene, and dimethyldisulfide (FD = 2048); hexanal and ethyl 2-methylbutanoate (FD = 1024); 2-methylthiophene (FD = 512); benzaldehyde (FD = 256); heptanol (FD = 128); 1-octen-3-ol, *t*-2-nonenal, and α-terpinolene (FD = 64); phenethyl alcohol (FD = 32); 1-octen-3-one, 2-undecanone, acetic acid, 2-methylbutanoic acid, and β-ionone (FD = 16). Additionally, ethyl hexanoate (DetF = 8), *t*-2-hexenal (DetF = 10), linalool (DetF = 9), nonanal (DetF = 9), myrtenol (DetF = 9), and β-pinene (DetF = 11) may contribute to 'Thornless Evergreen' flavor.

As determined on the polar (Stabilwax) column, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (FD = 1024); ethyl 2-methylpropanoate and 5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone (FD = 256); thiophene, linalool, 2-undecanone, hexanoic acid, 4,5-dimethyl-3-hydroxy-2(5*H*)-furanone and dimethylsulfide (FD = 128); cinnamic alcohol, 2-heptanone, *p*-methylacetophenone, and β-damascenone (FD = 32); 2-ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone, 1-octen-3-one and dimethyltrisulfide (FD = 16) may also be important to 'Thornless Evergreen' flavor. Additionally, diacetyl (DetF = 11), *l*-carvone (DetF = 11), methyl hexanoate (DetF = 10), allo-ocimene (DetF = 10), and α-pinene (DetF = 9), α-terpineol (DetF = 10), and octanol (DetF = 10) may contribute to 'Thornless Evergreen' flavor.

Since both cultivars were sampled identically for AEDA, their "comparative AEDA" can identify differences between their aroma components. Table 5.2 combines AEDA experimental results, and of 62 listed compounds, 31 had the same FD values (± 1 dilution) in both cultivars, implying comparable aroma impact. 'Marion' had seven compounds with FD

values higher than those of 'Thornless Evergreen', curiously only three were esters, and their average FD value was $2^{2.4}$. However, 'Thornless Evergreen' had 24 compounds with FD values significantly higher than those of 'Marion'; their average FD value was $2^{7.5}$, and included three esters, four ketones, and three furanones, which have intensely sweet, fruity, and caramel aromas. Additionally, 'Thornless Evergreen' had five sulfur compounds with intensely vegetal and alliaceous aromas.

Table 5.2: Flavor dilution summary of 'Marion' and 'Thornless Evergreen'

Compound[a]	Aroma descriptors this study	Stabilwax FD[b]		DB5 FD[b]	
		'Marion'	'T. Evrgrn'	'Marion'	'T. Evrgrn'
Acids					
Acetic acid[r]	acid, sour	2^4	2^5	-	2^4
Butanoic acid*[r]	rancid cheese, sour, pungent	$\mathbf{2^5}$	-	2^0	2^1
Hexanoic acid*[r]	pungent, sour	2^1	$\mathbf{2^7}$	-	-
2-Methylbutanoic acid*[r]	rancid cheese, sour, acid	2^5	2^7	2^5	2^4
Alcohols					
Benzyl alcohol[r] (T)	sweet, citrus, grass	2^1	2^1	2^0	2^1
Cinnamic alcohol[r] (T)	floral, tea, sweet, fruity	2^3	2^5	-	-
2-Heptanol[r]	woody, earthy, vegetal, minty	2^2	$\mathbf{2^9}$	2^3	2^1
Hexanol[r] (T)	floral, spice	2^2	-	-	-
Linalool[r]	sweet, floral, berry, green	2^2	$\mathbf{2^7}$	2^4	2^3
Octanol[r]	waxy, fruity	$\mathbf{2^1}$	-	-	-
1-Octen-3-ol	mushroom	2^2	-	-	2^6
Phenethyl alcohol[r]	floral, perfume, peach	2^3	$\mathbf{2^6}$	-	2^5
Aldehydes					
Acetaldehyde*[r]	grass, green	-	-	2^0	$\mathbf{2^3}$
Benzaldehyde[r] (T)	fruity, berry, juicy	-	-	2^6	2^8
Cinnamic aldehyde (T)	sweet, spice, cinnamon	2^2	-	-	-
Hexanal[r]	green, fresh	-	2^2	2^6	$\mathbf{2^{10}}$
t-2-Hexenal[r]	fruity, orange, green	-	-	2^0	2^2
Methional*	potato, earthy, onion	2^5	$\mathbf{2^9}$	2^8	2^{11}
3-Methylbutanal	fresh grass, fruity, leaf	-	2^2	2^0	2^0

Table 5.2 (continued): Flavor dilution summary of 'Marion' and 'Thornless Evergreen'

Compound[a]	Aroma descriptors this study	Stabilwax FD[b]		DB5 FD[b]	
		'Marion'	'T. Evrgrn'	'Marion'	'T. Evrgrn'
2-Methylpropanal[*] [(T)]	wood, grass	-	-	**2^0**	-
Nonanal[r]	floral, fruity	2^1	-	2^3	2^3
t-2-Nonenal[*]	watermelon, fresh vegetable, green	-	-	2^0	**2^6**
Esters					
Butyl acetate	fruity, juicy	2^1	-	-	-
Ethyl acetate[r]	floral, fruity	**2^4**	2^1	2^0	-
Ethyl butanoate	fruity, banana	2^2	-	2^1	-
Ethyl hexanoate[r]	fruity, floral	2^3	-	**2^5**	2^0
Ethyl 2-methylbutanoate[r]	fruity	2^2	-	2^7	**2^{10}**
Ethyl 3-methylbutanoate	fruit, sweet, banana	-	2^6	2^7	**2^{10}**
Ethyl 2-methylpropanoate[*]	sweet, fruity, berry, floral	-	2^8	-	**2^{11}**
Hexyl acetate[r]	fruity	-	-	2^0	2^0
Methyl butanoate	fruity, sweet	**2^2**	-	-	-
Methyl hexanoate[r]	fruity, green, sweet	2^3	2^2	-	-
Octyl acetate	floral, sweet	2^2	-	-	-
Octyl formate[*]	fruity	-	-	2^0	-

Table 5.2 (continued): Flavor dilution summary of 'Marion' and 'Thornless Evergreen'

Compound[a]	Aroma descriptors this study	Stabilwax FD[b]		DB5 FD[b]	
		'Marion'	'T. Evrgrn'	'Marion'	'T. Evrgrn'
Furanones					
2,5-dimethyl-4-hydroxy-3(2*H*)-furanone*[r]	fruity, sweet, caramel	2^{3}	**2^{10}**	2^{5}	-
4,5-dimethyl-3-hydroxy-2(5*H*)-furanone*[(T)]	spice, curry, fruity	2^{2}	**2^{7}**	2^{5}	2^{2}
2-ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone*[(T)]	cooked bramble, sweet caramel	2^{5}	2^{4}	2^{1}	2^{1}
5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone*[(T)]	roasted meat, cumin, maple syrup	2^{2}	**2^{8}**	2^{0}	-
4-hydroxy-5-methyl-3(2*H*)-furanone*[(T)]	caramel, strawberry, cooked bramble	2^{5}	2^{3}	2^{2}	-
Hydrocarbons					
Limonene[r]	overripe melon, green, tea	-	-	2^{2}	2^{1}
neo-allo-Ocimene*[(T)]	citrus, vegetal, cucumber	-	-	2^{4}	2^{2}
t-β-Ocimene*[(T)]	sweet, floral, woody, perfume	-	-	2^{3}	2^{1}
α-Terpinolene[r][(T)]	woody, sweet, earthy	-	-	2^{0}	**2^{6}**
Ketones					
2,3-Butanedione (Diacetyl)*[r]	buttery	2^{1}	2^{1}	2^{1}	2^{1}
l-Carvone	peppermint, fresh leaf	2^{3}	2^{2}	2^{1}	-
β-Damascenone[r][(T)]	sweet, floral, grape, blackberry	2^{2}	**2^{5}**	2^{3}	2^{2}
2-Heptanone[r]	fruity, banana, sweet, floral	2^{4}	2^{5}	-	-
β-Ionone[r]	floral, perfume, woody, spicy	-	-	2^{1}	**2^{4}**
p-Methylacetophenone	fresh, green, floral, fruity	-	2^{5}	2^{1}	2^{3}
1-Octen-3-one*[r]	mushroom, earthy	2^{3}	2^{4}	2^{1}	**2^{4}**
2-Undecanone[r]	floral, grn, pine, citrus	2^{3}	**2^{7}**	2^{2}	2^{4}

Table 5.2 (continued): Flavor dilution summary of 'Marion' and 'Thornless Evergreen'

Compound[a]	Aroma descriptors this study	Stabilwax FD[b]		DB5 FD[b]	
		'Marion'	'T. Evrgrn'	'Marion'	'T. Evrgrn'
Phenols					
Elemicin (T)	green tea, spicy, perfume	-	-	2^0	-
Eugenol[r]	woody, citrus, spicy	-	-	2^2	2^2
4-Vinylguaiacol*r (T)	BBQ rub, spicy	-	-	2^0	-
Sulfur					
Dimethyldisulfide*	vegetal	2^1	-	2^5	2^{11}
Dimethylsulfide* (T)	garlic bologna, cabbage	2^4	2^7	2^4	2^3
Dimethyltrisulfide*	vegetal, garlic	2^1	2^4	2^4	-
Methylethylsulfide* (T)	alliaceous, pungent	-	-	2^4	2^3
2-Methylthiophene* (T)	earthy, pungent	-	-	2^5	2^9
Thiophene* (T)	garlic bologna, sulfury	2^3	2^7	2^2	2^{11}
Theaspiranes					
Theaspirane A*r (T)	floral, earthy, tea, green	2^2	-	2^1	2^0
Theaspirane B*r (T)	earthy, fruity, sweet	2^2	-	-	-

a * = not previously reported in blackberry, r = reported in red raspberry, T = tentative identification

b FD = Flavor Dilution Factor; 'T. Evrgrn' = 'Thornless Evergreen'

The AEDA results mentioned above imply that 'Thornless Evergreen' has larger amounts of the 24 compounds, and they therefore contribute "more" to its aroma than to 'Marion's. However, the aroma descriptors of the esters, ketones, and furanones appear to be at odds with the descriptors for overall 'Thornless Evergreen' aroma, and much more appropriate to those of 'Marion'. This apparent dichotomy highlights the complex nature of aroma perception and analysis, and strengthens the case for aroma reconstitution analysis.

Table 5.3 summarizes cultivar aromas by compound class. The two cultivars have, with the exception of esters ("Marion' has 15, 'Thornless Evergreen' 8), comparable compound types and numbers, but with widely differing aroma impacts, as measured by flavor dilution (FD) factors. Fresh 'Marion' blackberry aroma has been described as floral, fruity, sweet, caramel-fruity, and woody, while fresh 'Thornless Evergreen' aroma is spicy, green, herbaceous, fruity, and sweet. Both cultivars contain the same numbers of odor-active acids, furanones, sulfur compounds, and Theaspiranes. The 'Marion' contains two more alcohols (hexanol, 2-methylpropanol), one more aldehyde (cinnamic), ketone (2-nonanone), and phenol (4-vinylquaiacol), three more hydrocarbons (β-myrcene, α-phellandrene, γ-terpinene), and eight more esters (benzyl acetate, butyl acetate, ethyl butanoate, methyl butanoate, methyl 2-methylbutanoate, nonyl acetate, octyl acetate, 2-phenylethylacetate) than 'Thornless Evergreen'. The 'Thornless Evergreen' has four alcohols (*cis*-3-hexenol, 3-methylbutanol, myrtenol, 4-terpineol) and one aldehyde (octanal), ester (ethyl 2-methylpropanoate), and hydrocarbon (camphene) not present in 'Marion'. Of thirty-seven newly reported volatiles, three organic acids, three aldehydes, five furanones, three hydrocarbons, three ketones, seven sulfur compounds, and two theaspiranes are shared by the cultivars (Table 5.3). This relatively large number of new volatiles is probably due to the extraction and

analytical methods used. It is thought that some portion of 'Marion' aroma is due to its hybrid pedigree, which contains at least 5 *Rubus* species, including raspberry (Finn et al., 1997). However, although forty-eight volatiles in this study have been previously reported in red raspberry (Georgilopoulos and

Table 5.3: 'Marion' and 'Thornless Evergreen' aroma by compound class

Compound[a] Class	Cultivar[b] Both (61)	'Marion' (16)	'T. Evergreen' (7)
Acids (pungent, sour, acid)	Acetic acid[r] Butanoic acid*[r] Hexanoic acid*[r] 2-Methylbutanoic acid*[r]		
Alcohols (floral, citrus, fatty, herbaceous, green)	Benzyl alcohol[r] (T) Cinnamic alcohol[r] (T) 2-Heptanol[r] Linalool[r] Octanol[r] 1-Octen-3-ol Phenethyl alcohol[r] α-Terpineol [r]	Hexanol[r] (T) 2-Methylpropanol* [r]	*cis*-3-Hexenol [r] 3-Methylbutanol [r] Myrtenol [r] 4-Terpineol [r]
Aldehydes (pungent, aromatic, green, floral, herbaceous, citrus)	Acetaldehyde* [r] Benzaldehyde[r] (T) Hexanal[r] *t*-2-Hexenal [r] 3-Methylbutanal 2-Methylpropanal* (T) Nonanal[r] *t*-2-Nonenal* 2-Octenal Pentanal	Cinnamic aldehyde (T)	Octanal
Esters (floral, fruity, sweet, citrus)	Ethyl acetate[r] Ethyl hexanoate[r] Ethyl 2-methyl/3-methylbutanoate[r] Hexyl acetate[r] Hexyl hexanoate* Methyl hexanoate[r] Octyl formate*	Benzyl acetate* [r] Butyl acetate Ethyl butanoate Methyl butanoate Methyl 2-methylbutanoate* Nonyl acetate* Octyl acetate 2-Phenylethylacetate*	Ethyl-2-methylpropanoate*

Table 5.3 (continued): 'Marion' and 'Thornless Evergreen' Aroma ...

Compound[a] Class	Cultivar[b] Both (61)	'Marion' (16)	'T. Evergreen' (7)
Furanones (strawberry-caramel, cumin, maple syrup)	2,5-dimethyl-4-hydroxy-3(2*H*)-furanone*[r]		
	4,5-dimethyl-3-hydroxy-2(5*H*)-furanone* [T]		
	2-ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone* [T]		
	5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone* [T]		
	4-hydroxy-5-methyl-3(2*H*)-furanone* [T]		
Hydrocarbons (herbaceous, citrus, pine, floral)	Limonene[r]	β-Myrcene*[r]	Camphene[r]
	allo-Ocimene*	α-Phellandrene[r]	
	neo-allo-Ocimene* [T]	γ-Terpinene[r]	
	t-β-Ocimene*		
	α-Pinene[r]		
	β-Pinene[r]		
	α-Terpinolene[r] [T]		
Ketones (strong, fruity, citrus, herbaceous, floral)	2,3-Butanedione (Diacetyl)*[r]	2-Nonanone*[r]	
	l-Carvone		
	β-Damascenone[r] [T]		
	2-Heptanone[r]		
	β-Ionone[r]		
	p-Methylacetophenone		
	1-Octen-3-one*[r]		
	1-Penten-3-one*		
	2-Undecanone[r]		
Miscellaneous (woody, fruity)	Theaspirane A*[r] [T]		
	Theaspirane B*[r] [T]		
Phenolics (spicy, clove)	Elemicin [T]	4-Vinylguaiacol*[r] [T]	
	Eugenol[r]		
Sulfur (vegetal, sulfurous, alliaceous)	Dimethyldisulfide*		
	Dimethylsulfide* [T]		
	Dimethyltrisulfide*		
	Methional*		
	Methylethylsulfide* [T]		
	2-Methylthiophene* [T]		
	Thiophene* [T]		

a (Class descriptors)

b * = not previously reported in blackberry, r = reported in red raspberry, T = tentative identification

Gallois, 1987a; Fenaroli, 1995; Nijssen et al., 1996; Roberts and Acree, 1996), thirty-five of them are common to both 'Marion' and 'Thornless Evergreen'; only eight are unique to 'Marion'. Fourteen volatiles out of 84 were described with aroma descriptors specific to bramble fruit (berry, blackberry, bramble, raspberry); no single compound was unanimously described as characteristically blackberry. In terms of total volatile composition, and based on total percentage of FID area, the 'Thornless Evergreen' contains significantly more alcohols, hydrocarbons, and phenols than the 'Marion'; the 'Marion' contains more acids and esters. Both cultivars contained comparable amounts of aldehydes and ketones, but alcohols were the most abundant volatiles. The six most abundant volatiles in 'Marion' were ethanol, acetic acid, hexanoic acid, ethyl acetate, linalool, and 2-heptanol; they totaled 52% of total peak area. In 'Thornless Evergreen' the six most abundant volatiles were 2-heptanol, ethanol, 2,3 butanediol, hexanol, α-pinene, and ethyl acetate; they totaled 43% of total peak area.

The parallel use of P&T-DetF GC-O and SAFE-AEDA provided more complete and representative blackberry volatile compositional data, and useful comparisons of the cultivars' aroma profiles. Although 'Marion' and 'Thornless Evergreen' blackberries have many potent odorants in common, qualitative aroma comparisons consistently note the more floral, caramel-fruity, sweet aroma of 'Marion' compared to the spicy, herbaceous, less fruity aroma of 'Thornless Evergreen'. The significant odor-active compounds identified in these cultivars support the perception that characteristic blackberry aroma is a complex formulation of volatiles, rather than a simple mixture of a relatively small number of character impact compounds. All intensity measures obtained in GC-O are generally useful, but are approximations of the sensory relevance of odorants (Guichard et al., 1995;

Blank, 1997). Regardless of method, GC-O results give indications of the potency of odor active volatiles, but not final conclusions of their sensory relevance (Blank, 1997). GC-O methods do evaluate odorants out of context, as the actual smell of blackberries is a complex function of the nasal and retronasal stimuli generated from the fruit matrix. Accordingly, a compositional analysis to determine true blackberry aroma must include the analytical quantification (i.e., OAVs) of all odor active volatiles suspected to be significant to that aroma (Grosch, 1994; Buettner and Schieberle, 2001a, 2001b). This quantification data will generate blackberry aroma reconstitution studies (Buettner and Schieberle, 2001a, 2001b; Ferreira et al., 2002), which are the best, most efficient use of trained sensory panels.

Aroma reconstitution studies verify collected analytical data, and correct for the limitations of GC-O intensity and threshold determinations (Mistry et al., 1997). Aroma reconstitution produces formulations of suspect odor active volatiles that are compared to the original food sample. Through repeated testing and alteration of formulations, panels of trained assessors can develop and identify an aroma formulation that matches original blackberry aroma sensory attributes. Research data implies some portion of the more floral, fruity, and sweet aroma of the 'Marion' blackberry may be the result of additional esters not shared with the 'Thornless Evergreen' blackberry, yet both cultivars apparently contain five furanones, which are powerful sources of sweet, fruity, and spicy aromas. Aroma reconstitution studies will be the key to resolving the significant aroma profile differences between 'Marion' and 'Thornless Evergreen' blackberries.

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